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REGENERATION OF BONE.

AN ESSAY, ILLUSTRATED BY PHOTOGRAPHS AND PHOTOMICROGRAPHS, DEALING WITH THE REPAIR OF SIMPLE FRACTURES AND THE REGENERATION OF BONE AFTER PARTIAL INJURY AND SUBPERIOSTEAL RESECTION.

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(From the Laboratory of Surgical Pathology of the University of Pennsylvania.)

INTRODUCTION.

THE subject of regeneration of bone, especially with reference to the repair of fractures, has occupied the attention of scientific writers from the earliest days of medical history to the present time. Research along this line was especially active in the latter part of the eighteenth and the early part of the nineteenth century. It was revived by the work of the naturalist Duhamel du Monceau, who called attention to the active part played by the periosteum in the repair of bone and later by the enduring work of the great French surgeon, Dupuytren, who placed the subject upon a scientific basis. In spite of the tremendous amount of labor that has been devoted to the investigation of this subject, there has never been even a fair amount of unity of the opinions of the writers upon the subject. As far as I am able to judge, about the only subject on which everyone agrees is the fact that the bone unites. As to which portion or portions of the bone are responsible for that union there are almost as many opinions as there are writings, so that one is left in a maze when he endeavors to arrive at conclusions by reading the literature.

Being cognizant of the above and at the request of Dr. Frazier, the writer was induced to take up the subject in order to endeavor to formulate an opinion that would be satisfactory to himself and to try to clear up some of the haze that surrounds the subject. In almost all writings upon this matter, the authors have begun by giving a chronological account of the work done by others and then proceeding to combat the facts contained in these writings. In view of the fact that there are already so many sources from which to obtain this knowledge, and since this paper is nowise based upon work done by any writer, with the possible exception of Dupuytren and Nichols, it is deemed unnecessary to lengthen the article by repetition, but it is proposed to let the paper itself present the writer's findings and deductions. Very little reading upon the subject had been done previous to the completion of the work and the formulation of opinions, so that the writer should not be biased in his views, thereby working according to preformed ideas. Due attention will be given to the work of others in the portion of the paper devoted to conclusions, where the opinions of the most important writers will be compared with the views contained in the paper.

Before proceeding with the body of the paper, I wish to express my gratitude to the authorities of the University of

Pennsylvania, especially Dr. Chas. H. Frazier, for the priviledge of conducting the experiments in their laboratories and for the abundant facilities and material they placed at my disposal. I also take this opportunity to thank all who have by their advice, encouragement, or assistance aided me in bringing this study to a successful conclusion.

SYNOPSIS OF EXPERIMENTS.

The work of experimentation has covered a period of two years, during which time about 135 experiments have been performed upon rabbits and dogs. This number has been decreased considerably by loss through infection (very great in the beginning, due to poor facilities which were improved later), and owing to the fact that some of the animals and gross specimens were lost. No experiment has been included in which there was non-union of a well-recognized degree. In addition, about fourteen rabbit experiments were used in developing the proper technique for grinding the specimens. As a result of the losses from various sources, this paper is based on the reports of sixty-four dog and seventeen rabbit experiments.

The experiments have been divided into fifteen series, each for the purpose of determining the individual activities of certain portions of the bone independent of other factors. The different series, with their purposes, were as follows:

SERIES 1. Upon rabbits, to study complete fractures from the first day until about the thirty-fifth day, to determine the action of all the tissues from the earliest period until the full development of the callus. The technique was carried out in such a manner (see Technique) that none of the soft tissues were removed, but the region of the fracture studied in its entirety.

SERIES 2. Upon dogs, to study the later developments in the callus during the process of union. The experiments consisted in making complete fractures of the tibia.

Series 3. Upon dogs, to study the result of injury to all portions of the bone, independent of extrinsic forces. In this series, an effort was made to cause a complete fracture of one-half of the bone, leaving the other half intact in order to maintain perfect position and prevent any mobility of the ends of the fragments.

Series 4. Upon dogs, to determine the activity of the medulla and cortex independent of the periosteum. It was a repetition of the last series with the exception that the periosteum was removed in the region of the fracture.

Series 5. Upon dogs, to determine the activity of the cortex independent of the periosteum and the medulla. The experiments consisted in removing the periosteum and making superficial injuries to the bone without entering the medullary cavity.

Series 6. Upon dogs, to study the activity of the periosteum raised from the bone, when accompanied by injury to the bone not sufficiently deep to enter the medullary cavity.

Series 7. Upon dogs, to determine whether an animal substance placed under the periosteum, when not accompanied by injury to the bone, would cause the formation of osseous tissue. Catgut was the substance used.

Series 8. Upon dogs, to determine whether an unabsorbable foreign material, placed under the periosteum, without additional injury to the bone, would excite the formation of new osseous tissue. In this instance celloidin hardened in bichloride alcohol was used.

Series 9. Upon dogs, to determine the influence, upon osteogenesis, of blood clot under the raised periosteum, when not accompanied by injury to the bone.

Series 10. Upon dogs, to determine the effect of merely raising the periosteum, with as perfect hemostasis as possible and without injury to the bone.

The next four series were performed with the object of determining the possibility of regeneration of the tibia after

Nichols' subperiosteal resection, when not in the presence of inflammation. They were as follows:

Series 11. Upon dogs, to determine the influence upon regeneration exerted by allowing a small bridge of osseous tissue to remain under the periosteum, instead of removing the entire section of bone.

Series 12. Upon dogs, to determine the activity of the periosteum alone. These operations consisted in typical subperiosteal resections.

Series 13. Upon dogs, to determine the influence exerted upon regeneration by the periosteum, when the medulla is replaced between its layers after the removal of the cortex.

Series 14. Upon dogs, to determine the influence upon regeneration exerted by bone salts deposited between the layers of the periosteum, after the removal of the cortex.

Series 15. Upon rabbits, to determine, if possible, what role the fibrin took in the formation of cartilage. The experiments consisted in making complete fractures and, at very short intervals afterward, exposing the area injured and making smears and sections from the callus. Unfortunately the results of this series are negative, owing to defective technique.

TECHNIQUE.

In the first, or rabbit series, in which complete fractures were made without incision of the skin, an effort was made to maintain the relations just as they were at the time of death without removing the tissues surrounding the site of fracture. To accomplish this two methods were employed: First, the entire leg, denuded of skin, was placed on the stage of a freezing microtome and frozen hard. The entire leg was then cut through at a fair distance from the site of fracture and the tissues placed in Müller formol for several days, to harden before decalcification. Later it was found that freezing was not necessary, so, in its stead, the specimens were immediately placed in Müller formol and after a day or two they were found hard enough to cut across in the manner described in the freezing method. This method was used with the later specimens. After cutting the specimens and leaving them in Müller formol for another day or two to harden further, they were washed for twenty-four hours in running water and then placed in the decalcifying fluid.

The decalcifying fluid which was used for the rabbit experiments and the earlier dog experiments consisted of nitric acid 5 per cent., about 1 per cent. formalin, 5 per cent. of a saturated aqueous solution of picric acid, and water to make 100 per cent. This fluid was not as good as the one which will be given later under the technique of the dog experiments, as it does not give good nuclear staining.

The specimens were decalcified until they could be penetrated with a pin and were then washed for several days in running water, 95 per cent. alcohol for several days, absolute alcohol for twenty-four hours, absolute alcohol and ether for twenty-four hours, and three strengths of celloidin. After three weeks' embedding in celloidin they were mounted and cut on a Minot precision microtome. These specimens were not as good as they might have been had the fluid used in the later dog experiments been used, and had they been washed in alcohol until all yellow tint had disappeared from the wash alcohol after standing twenty-four hours. This is a very important step, as the water is not so effectual in removing the picric acid as is the alcohol, and unless every trace of it is removed the picric acid interferes with the embedding and, very materially, with the staining, the tissues either refusing to take the stain or losing it very promptly afterward. The acid also acts on the celloidin so that it refuses to harden in the mounting of the specimens. The embedding of the deeper portions of the specimens might have been better had the dense outer layer of deep fascia been removed so that the celloidin could more easily penetrate the tissues

The technique in the dog experiments differed somewhat, owing partly to the fact that the specimens had to be divided to be embedded in two different manners, and partly as the result of the recognition of the defects in the process as originally carried out. The animals were killed with chloroform or gas and the legs were immediately amputated and prepared for fixing. The conditions of the wound were noted and then the skin carefully removed so as not to interfere with the operated area of the bone. Muscular tissues were removed and a section of the bone retained, which went wide of the site of the operation in order to include all possible callus or changes in the bone as the result of injury. This section was immediately placed in 50 per cent. formalin, although 20 per cent. formalin was used on several occasions without apparent difference in the results. In three or four days, depending on the size of the section, it was removed from the formalin, washed in water for a time, placed in a vise and sectioned longitudinally with a specially devised saw, bisecting the operated area and the medullary cavity. This was done so that a section could be run through the celloidin embedding, and another section run through the balsam embedding for grinding. A description of the macroscopic appearances was noted and the two sections again placed in 4 per cent. formalin to fix whatever portions of the medulla may have escaped fixation in the several previous days. After several days of rehardening the specimens were washed in water for twenty-four hours and then one-half was run through celloidin and the other half through balsam embedding.

The saw mentioned above was on the principle of the jeweller's saw, only a little firmer and about five-eighths inch wide to maintain a straight direction as it went through the bone. Alternate teeth were deviated to one side or the other to prevent jamming of the saw in the deeper portions of the cut.

The specimens to be cut in celloidin were immediately placed in the decalcifying fluid, which originally was that used in the rabbit experiments. Later it was found that the efficacy of the fluid could be greatly enhanced by the addition of 15 per cent. alcohol (95 per cent.). When this fluid is used, very good nuclear staining can frequently be obtained. When the specimen was penetrable with a pin it was washed in running water for two or three days, followed by alcohol, which was changed daily until there was not the slightest tint of yellow in the alcohol on standing twenty-four hours. The specimen was then run through absolute alcohol, absolute alcohol and ether, and, after being in two or three thicknesses of celloidin for three weeks, was mounted and cut. The thickness of the cut sections varied greatly. All were stained with hematoxylin and eosin.

BALSAM EMBEDDING. In order to see what could be learned from specimens ground to microscopic thinness without previous decalcification, one-half of each bone was run through a process of embedding after the style of Weil's process, but with modifications, the result of previous experimentation. The specimens were hardened in formalin in the manner described, washed in water, and then again hardened in 95 per cent. alcohol for a few more days. Following this they were rinsed off in water and placed in Grennacher's borax-carmine stain. They were left in this solution eight days, as it is very slow-acting, and specimens cannot be overstained with it. After this the sections were placed immediately in acid alcohol (1 per cent. hydrochloric acid in 70 per cent. alcohol) without preliminary washing in water.

They were allowed to stay in this solution for about forty-eight hours, then transferred to 95 per cent. alcohol for twenty-four hours, absolute alcohol twenty-four hours, cedar oil twenty-four to forty-eight hours, xylol for a few minutes, and then a thin solution of xylol balsam. This xylol balsam is not the usual balsam used in mounting specimens on slides, but is made by first evaporating paper-filtered Canada balsam to dryness, to rid it of turpentine, and then adding this hardened balsam to xylol.

The best dish in which to evaporate balsam has been found to be a sheet-steel sand-bath. This will not rust readily, is more or less pliable, and, in addition, does not break in the effort to remove the balsam. If a porcelain dish is used the balsam must be dug out with a sharp knife or instrument, and the chances are strongly in favor of penetrating the bottom of the dish. With the sheet-steel one, by tapping omtthe bot of the dish and bending in the sides, the balsam can be removed as a single large mass and later broken into fine pieces.

The specimens were allowed to remain in thin balsam for a few days and then each day a little powdered balsam added until, at the end of a week or so, the xylol was saturated with it. In a couple of days the specimens were ready for evaporating. This was done by placing them in shallow watch-crystals, with the important area downward, covering the specimens partially with saturated xylol balsam. The watch-crystals were placed on a sand- or water-bath for evaporation. This is a very slow and tedious process, as the water should not go above 95° C., for otherwise the specimens will be cooked. Where there are facilities for constant observation, and where there are not many specimens to be evaporated, the water-bath is the better of the two, as the temperature can be more easily regulated and the specimens be evaporated more quickly. The sand has a tendency to get too hot or too cold, and is rather hard to regulate. Even when the temperature of the sand is higher than the water it does not appear to do the work so well. The sections were identified by labelling the crystals before pouring on the balsam. The balsam protects the labels and the writing is visible through the transparent mass.

When the specimens had become so hard that the balsam, when cooled, fractured on the insertion of a sharp instrument, they were ready for grinding. The balsam had to be brittle, for otherwise the grinding on the rapidly moving disk would have heated it and made it drag, not grinding off clean and sharp. The specimens were readily removed from the crystals by melting a thin layer of balsam on the bottom by passing it through a Bunsen flame. This allowed the entire mass to be pushed off and rapidly cooled under running water to cool the mass before the balsam around the specimens also became soft.

The machine used in grinding the specimens was a modification of the machine devised by Dr. Frederick Allen, of the Dental Department of the University of Pennsylvania. The modifications are unimportant, being merely the addition of another drip spigot on the opposite side, so that the grinding surface should never become dry, and the widening of the drip pan to save rusting of the machine. An accurate description of the machine can be had by referring to the original article of Dr. Allen. The idea of the machine is to afford a means of controlling, with comparative accuracy, the thickness of the specimens to be ground by means of mathematically accurate screws, etc., to control the platens and grinding disks. It is a vertical lathe of very fine adjustment. Mr. Cowarden, of Richmond, Virginia, has ground some very fine sections of bone with the soft parts detached by means of a horizontal lathe of cruder construction.

The grit that gave the greatest satisfaction was finely powdered glass. Pumice and carborundum were tried, but both leave the specimens, especially the soft tissues, in an extremely dirty condition. By means of a very thin suspension of powdered glass, sections have been obtained which are practically free from dirt. I believe that this result has not been obtained hitherto by other means.

The specimen to be ground was held by the fingers against the rapidly moving disk, upon which a watery suspension of powdered glass was placed with a brush, until a flat surface was obtained and the specimen reached. It was then ready to be placed on the platen. This was done by means of balsam that had been hardened until it would fracture on the introduction of a pin, but not so hard and brittle as the balsam around the specimen, for if this balsam were made too brittle it would lose some of its toughness and the specimen would readily come off the platen during the process of grinding. A thin layer of balsam in the liquid state was obtained by heating the platen with powdered balsam upon it. The specimen was placed firmly on it and rapidly chilled by placing it under running cold water. The platen was then placed in the machine and the disk run with the powdered glass on it until the specimen was ground down to the particular area desired for study, when no more glass was added, but the disk kept running with a liberal supply of water on it to wash out whatever glass had been caught in the meshes of the bone.

The one side was then completed, but the section may still have been very thick or the side that had been ground by hand not very even. Therefore, it had to be transferred to another platen to grind the other side. Before this was done the zero point for the platen to be used was determined on the machine. Zero is the point at which, when swinging the arm of the upright platen holder, the platen will just barely escape the grinding disk. When this was determined, an indicator was screwed into place at the zero point on the screw index.

The platen was then removed, covered with a thin layer of melted evaporated balsam, the ground surface of the specimen on the original platen forced into this film so that the rims of the two platens coincided, and the whole thing placed under running cold water. This left the two platens united by the section between. To remove the first platen it was held in the flame, the heat of the metal soon melting a thin film of balsam on its surface without affecting the section. It could now be easily removed, leaving the specimen on the second platen. After again cooling it was ready for final grinding.

The grinding was done rapidly until about thirty points from zero was reached, when caution was observed and the specimen frequently inspected to determine its thickness. The machine was supposed to give the exact thickness, but the varying thickness of the layer of balsam beneath and of the amount of grit placed on the machine made the eye a better judge of the thickness than the screws. I have lost a number of beautiful specimens by giving the screw just another turn and finding my section ground to nothing.

When the specimen was as thin as desired and had been washed by allowing the machine to go with nothing but water upon the disk, it was ready to be removed and mounted. This was done by moistening it with gasoline upon a towel and then pouring thin celloidin over it. This was either hardened in alcohol or allowed to harden in the air. The platen was then immersed in benzole until the specimens dropped off, to be followed by another change of benzol to remove the excess of balsam that might still remain, carbol-xylol to clear the celloidin, xylol, placed on a slide and covered with xylol balsam and cover-slip.

The illustrations are, for the most part, photographs of the original specimens. All the microscopic pictures, with the exception of one, are photomicrographs of the specimens themselves taken by Mr. Louis Schmidt. This one and one gross picture are black and white wash drawings made by him. The radiographs are positives made from negatives of the specimens with all the soft parts attached. They were made by Dr. Pancoast, of the University Hospital. The radiograph of the human ulna was made by Dr. Newcomet, of the Presbyterian Hospital.

COLLECTIVE REVIEW OF INDIVIDUAL SERIES.

SERIES 1.

In this series the rabbits were placed under the influence of an anesthetic, usually ether (with the occasional use of chloroform), the foreleg grasped between the fingers and broken. The legs were then wrapped in cotton and a sodium silicate bandage applied. It was extremely difficult to keep the dressing on the animals, it being frequently necessary to reapply them several times. Grossly, from the third to the eighth day, there was no union of the fragments, but the mobility did not appear to be as great as when the leg was first fractured. Apparently, there seemed to be something gluing the fragments together without giving the impression of distinct union. On the ninth day there was an absence of lateral mobility, but there was some rocking of the fractures distant from the site of the fracture. From the twelfth to the eighteenth day there was fair union of the fragments, and from that time on the union was good.

Microscopically, most of the specimens, with the exception of the greenstick fractures, show considerable fragmentation of the ends of the bone and displacement of the fragments. Even from the first day the specimens present a remarkably small amount of free corpuscular blood and none was noticed after three and a half days. There was only a very moderate leukocytic infiltration noticed, and none at all after three and a half days. The feature to which especial attention should be called, and concerning which I have seen practically no mention, is the abundance of fibrin in the region of the fracture. This is present on the first day as a loose network. By the second day it begins to form dense masses, in addition to finer networks containing corpuscular blood in its meshes. By the fourth day it is present as compact masses or streams coursing between the ends of the fragments (Fig. 1). It continues to be present in the form of masses until about the sixth day, when it disappears gradually until about the tenth day, although vestiges of it were found as late as the fourteenth day.

As early as the second day the periosteum and endosteum exhibit signs of proliferation. The cells of the periosteum multiply, with the formation of three or four layers of spindle cells having oval, vesicular nuclei. A formation of a similar layer of cells distant from the site of the fracture occurs also in the endosteum, with the exception that some of the cells are slightly more polygonal in shape. The blood appears to have lacerated the medulla farther up on the side of the bone than in the centre. The medullary tissue shows an increase of connective tissue between the fat cells, with a commensurate decrease in the number of these cells. The muscular tissue is infiltrated with blood and a fine network of fibrin. It is beginning to show evidences of connective-tissue hyperplasia, young fibroblasts being present between the fasciculi here and there throughout the muscular tissue.

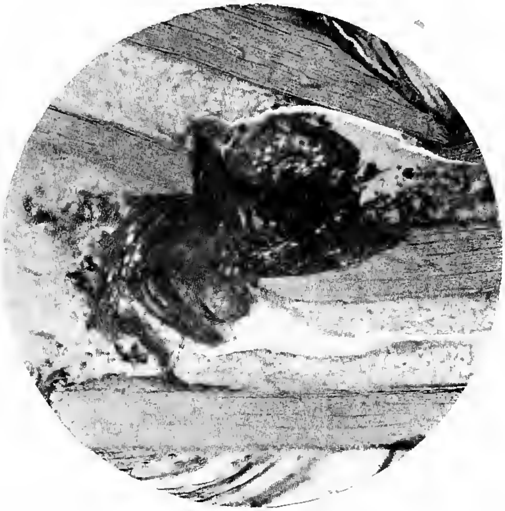

Fig. 1.—Experiment 5, Series 1, fracture four days. Shows lateral displacement of the fragments and dense "streams" of fibrin coursing between their ends.

This process continues for the next two days without any marked changes taking place. On the fourth day differentiation occurs in the periosteum and endosteum at some distance from the site of fracture. The periosteum exhibits considerable hyperplasia of both its superficial fibrous layer and its deeper osteogenetic layer, with the formation of large polygonal cells having large vesicular nuclei. At some distance from the site of fracture the cells appear to fuse, or the ground substance between the cells to become more hyaline and transparent, the whole taking on a homogeneous red stain with eosin, in the form of lines running vertical to the surface of the cortex. These bridge over to meet each other a short distance from the cortex (Fig. 2). Some of the cells are included in the mass and nothing but their nuclei is apparent. Further experiments demonstrate that this is only apparent, since a small amount of protoplasm is included with the nucleus, but greatly compressed by its

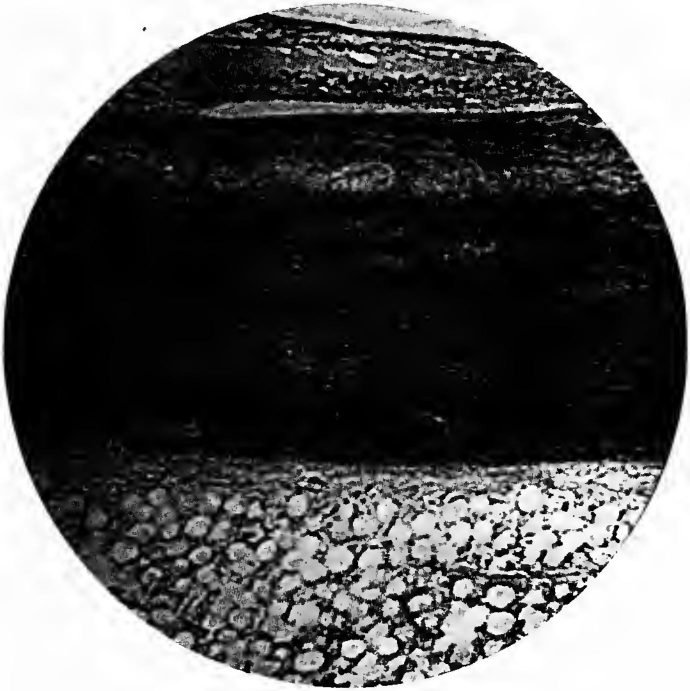

Fig. 2.—Experiment 5, Series 1, fracture four days. Illustrates the earliest formation of trabeculæ in the periosteum. The picture includes one side of the cortex with the periosteum above and the medulla below. The new trabeculæ are seen to bridge over to meet each other.

confined quarters. The above is the first evidence of osteoid tissue in the periosteum, and particular attention is called to the fact that this transformation occurs at some distance from the line of fracture. Nearer to the ends of the fragments the cells of the periosteum appear to be embedded in a more or less homogeneous groundwork which does not take the eosin staining and which is without definite arrangements of the cells, the whole having more the appearance of forming cartilage than of anything else. Attention is called to the fact that this occurs nearer the masses of fibrin than does the osteoid tissue.

While the above is taking place in the periosteum, a similar process is occurring in the medullary cavity The endosteum is also showing proliferation, with the formation of the same large polygonal cells having a small amount of transparent substance between the cells. This substance apparently increases, as in the periosteum, and fuses with the protoplasm of the cells, the whole again assuming a homogeneous red staining, in lines, with the formation of the trabeculæ. These project from the inner side of the cortex in a manner similar to the periosteal trabeculæ on the outside-This process, also, occurs at some distance from the site of fracture. In the medulla there is a continual increase in the connective-tissue hyperplasia between the fat cells. The muscular tissue exhibits an increased amount of young connective tissue between the fasciculi.

After the fourth day there is always abundant osteoid tissue on the periosteal aspect and also considerable amount in the medullary cavity, though less than in the periosteum. It continues increasing in the periosteum until the ninth day, when osteoclasts make their appearance in the form of large polynuclear cells, which apply themselves to the sides of the trabeculæ. These, by some chemical or phagocytic action, dissolve out the lime salts and destroy the trabeculæ. Their mode of action will be taken up later. These cells, from the ninth day on, continue to absorb the newly formed trabeculæ, while new deposits by osteoblasts are occurring in other places. Their early appearance is probably with the purpose of forming new channels or spaces more in conformity with the line of the bone. The medullary trabeculæ have undergone but little absorption in any of the specimens. From the twelfth day there is absorption of the cortex, increasing commensurately with the duration of the fracture.

The line of fracture and the deposit of cartilage will next be considered.

As stated before, until the tenth day the material between the fragments consists mostly of fibrin and detritus, with fragments of bone, etc. From the fourth day onward, on the periosteal side of the fracture, there can be noted varying amounts of cartilage. Some of it is hyaline in character with very large cells, having even, in a few cases, a twin arrangement. Other specimens contain fibrocartilage. In some areas there appears to be almost a transition from fibrin into cartilage through invasion by connective tissue, with the conversion of these connective-tissue cells into cartilage. The appearances are not conclusive, but suggestive. On the outskirts of the cartilage are seen distinct areas of transition into osteoid tissue, the ground substance increasing in amount and assuming a red color while the cells shrink. This occurs in certain streaks, leaving, in areas, solid islands of cartilage between. In other locations these islands, or at least the ground substance of them, appear to have been absorbed with the liberation of cells, accompanied by penetration of bloodvessels and connective tissue, forming marrow cavities between the trabeculæ. By reference to chart No. 1, it can be seen that the cartilage appears between the ends of the fragments as the fibrin disappears, one specimen showing the two existing together across the line of fracture. The close proximity of the cartilage to the small amount of fibrin that is still remaining between the ends of the fragments is well illustrated by Fig. 3, from a fracture of eight days' duration, which was merely greenstick.

The fact that cartilage is not a growth from the periosteum alone is demonstrated very clearly by the same figure. In this instance some of it is to be seen in the medullary cavity. It could hardly be from a shred of periosteum left in the medullary cavity, for the fracture was so slight that there was not sufficient displacement to allow a shred of periosteum to become detached and left there.

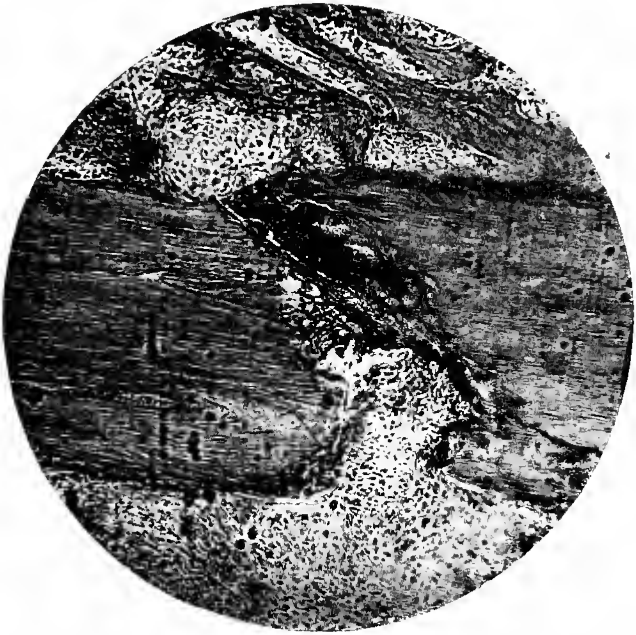

Fig. 3.—Experiment 9, Series 1, fracture eight days, greenstick. Demonstrates the close proximity of the remnant of fibrin between the fragments to the small masses of cartilage on the periosteal (above) and medullary aspect of the cortex.

Thus we find that the tissue, holding together the fragments and spindles of osteoid tissue, giving the impression that the fracture is entirely healed, is cartilage and not bone at all, for in none of the experiments up to thirty-five days are there any osteoid trabeculæ to be found between the ends of the fractured cortex.

The typical appearance of a fracture which has been maintained in good position and in which there has been nothing to interfere with union is seen in Fig. 4. It shows well the internal and the external splinting of the sides of

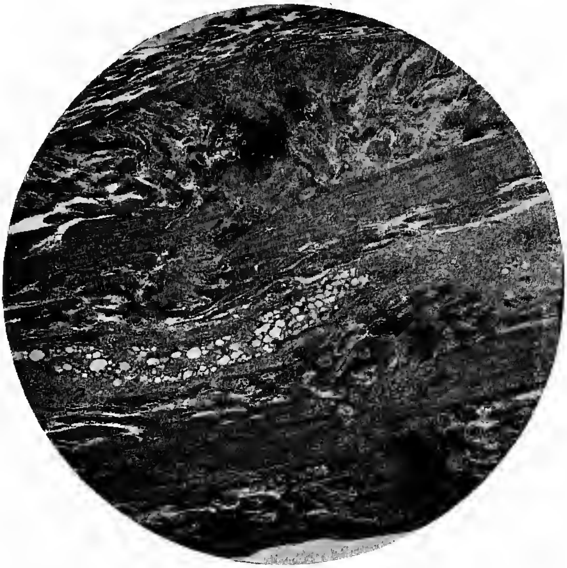

Fig. 4.—Experiment 12, Series 1, fracture twelve days. Illustrates the endosteal and periosteal splints that maintain the position of the fragments. There are small islands of cartilage in the periosteal splints at the line of fracture, but there is only fibrous tissue between the ends of the fragments.

the cortex with periosteal and medullary trabeculæ; shows the hyperplastic condition of the marrow, which is continuous in a narrow channel between the two spindles of medullary callus; and shows that between the fragments themselves there is connective tissue and cartilage, but no osteoid tissue. This figure also well illustrates the osteoporosis that occurs in the ends of the fragments forming communications between the intertrabecular medullary spaces and the old Haversian canals.

The amount of displacement and mobility of fragments determines, to a great extent, the size of the callus and the rapidity of final union. This fact is demonstrated by a greenstick fracture in which there had been, probably, very little displacement, and in which in the course of twenty-one days had proceeded to almost complete union. It shows well the external splint of dense osteoid trabeculæ in which there is absorption of the deeper portions and also illustrates the absorption of the cortex. There do not appear to be many osteoid trabeculæ remaining in the medullary cavity. Osteoclasts are present in abundance. Between the ends of the cortex there still remains connective tissue and not bone, showing that the process is not as yet complete.

Before concluding this series there remain some isolated facts to be stated. In the first place, in some of the fractures which appear to be fairly well united, the ends of the bone do not seem to exhibit union with any character of tissue, but appear to lie loose in the tissue between the fragments and to be held in place merely by the external cartilage splints. In the red hypertrophic marrow of the bone there are numerous large cells with a large amount of protoplasm, and very large nuclei which show a tendency to divide in some cases. These cells are probably the osteoclasts in the process of formation or in a state of inactivity. Finally, there is little tendency to absorption on the part of the detached fragments but more frequently there is a deposit of osteoid tissue on the surface. Eighteen successful rabbit fractures were obtained in this series.

SERIES 2.

For the sake of making investigation complete and of studying the later changes that occur in the callus (in the line of Dupuytren's idea) Series 2 was performed. Dogs were used because of their more robust nature and because of the fact that they can be more easily dressed and the relations of the fragments better maintained. In each case the animal was etherized, the foreleg grasped firmly between the two hands and fractured over the side of the table. The fracture was then reduced and a shaped wooden splint applied, after a liberal application of cotton, the splint being held in place by a sodium silicate bandage. The animals were killed at periods varying from twenty-one to two hundred and seventy-seven days, the latter being the longest time in the experiments. Nine legs were broken in this series.

Examination of Chart 1 (of the rabbit series) and Chart 2 (Series 2) demonstrates how closely the condition of the repair in the two classes of animals coincide. Almost the same result has been obtained in the rabbit of eighteen days and the dog of twenty-one days, with the exception that the dog leg is not so firm, there being more weight to be carried, the animal being of a larger species. In both, osteoid trabeculæ are present in the periosteum and in the medullary cavity, but none between the fragments. In both, there is absorption of the periosteal trabeculæ and cortex, with questionable, if any, absorption of the medullary trabeculæ. Both show hyperplasia of the marrow and cartilage in the periosteum, with cartilage and connective tissue across the entire line of fractures. With this close relationship between similar fractures in the two species, the series can be considered continuous and the results in one species applied to the other. The dog experiment even shows a small remnant of fibrin on the outskirts of the cartilage and connective tissue separating it from the bone. All the fractures of this series appear, clinically, to be firmly united, excepting two in which there was slight rocking mobility.

CHART 1.

Number.	Series.	Days.	Free blood between fragments.	Leukocytes.	Fibrin between fragments.	Trabeculæ in			Absorption of			Connective-tissue hyperplasia of medulla.	Cartilage in			Changes in muscles.	Apparent union.
						Periosteum.	Between fragments.	Medullary cavity.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.		Periosteum.	Between fragments	Medulla.		
1	1	1	Lit.	Mod.	Loose	—	—	—	A	A	—	—	—	—	—	Fragmentation.	—
2	1	2	—	Few.	Netw. Mass.	—	—	—	A	A	—	+	—	—	—	Free blood, fibrin.	Greenstick (?).
Greenstick.																	
3	1	3	Lit.	—	Mass.	—	—	—	A	A	—	+	—	—	—	Fibroblasts.	Very slight
4	1	3½	Lit.	Few.	Netw. Mass.	—	—	—	A	A	—	?	—	—	—	Fragmentation, blood.	?
5	1	4	—	—	Netw. Mass.	B	—	+	—	—	—	+	+ ?	—	—	Conn. tiss.	—
6	1	5	—	—	—	+	—	+	—	—	—	+	+	+	—	Conn. tiss.	Slight.
7	1	6	—	—	Mass.	+	—	— ?	?	?	+	+	+	—	—	?	Slight.
8	1	7	—	—	+	+	A	+	—	—	—	+	—	—	—	—	
Bone forceps.																	
9	1	8	—	—	Lit.	+	—	+	—	—	Lit.	+	+	—	+	—	Greenstick.
10	1	9	—	—	Lit.	+	—	+	+	—	—	+	+	—	—	Conn. tiss.	Rocking.
11	1	10	+	—	+	+	A	+	—	—	—	+	+	—	—	—	
Bone forceps.																	
12	1	12	—	—	—	+	—	+	+	+	+	+	+	Lit.	+	—	+
13	1	13	—	—	—	+	—	+	+	—	+	+	+	+	—	?	—
14	1	14	—	—	+	+	—	+	+	—	+	+	+	+	—	Conn. tiss.	Fair.
15	1	18	—	—	—	+	—	+	+	—	+	+	+	+	+	Conn. tiss.	+
16	1	21	—	—	—	+	—	+	+	+	+	+	—	—	—	?	+ greenstick.
17	1	24	—	—	—	+	—	?	+	..	+	..	..	+	..	....	+
18	1	35	—	—	—	+	—	Lit.	—	—	—	+	+	+	—	Conn. tissue.	+

Explanation: + present. — absent A, tissue of origin absent. Lit., little. Mod., moderate. Mass., present in masses. Netw., present as a network. Conn. tiss., connective tissue. ? doubtful. B, beginning.

CHART 2.

Number.	Series.	Days.	Trabeculæ in			Absorption of			Connective-tissue hyperplasia of medulla.	In periosteum.	Between fragments.	In medullary cavity.	Apparent union.	Dense connective tissue between fragments.
			Periosteum.	Between fragments.	Medullary cavity.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.						
19	2	21	+	—	+	+	—?	+	+	+	+	+	Some mobility.	+
20	2	33	+	—	+	+	+	+	. .	+	—	—	+	+
21	2	57	+	—	+	+	+	+	—	+ ∧	+	—	+	+
22	2	63	+	—	+	+	+	+	+	+ ∧	—	—	+	+
23	2	85	+	Few.	+	+	—	+	+	+ ∧	+	+	+	—
24	2	88	+	—	+	+	+	+	+	+ ∧	+	+	Very slight rocking.	—
25	2	129	+	+	+	+	+	+	+	—	—	—	+	—
26	2	262	+	+	+	+	+	+	?	+ ∧	—	—	+	—
27	2	277	+	+	+	+	+	+	?	+	+	—	+	+

Explanation: + present. — absent. ? doubtful. ∧ wedge-shaped.

Until we reach fractures of very long standing, cartilage in the periosteal callus was found to be present constantly. The amount of this callus and its shape was found to be dependent on the amount of lateral and especially angular displacement. In the fractures of twenty-one and thirty-five days (Fig. 5), there was no angular displacement, so that the cartilage was present merely as a line on the periosteal aspect of the line of fracture. Later on in the series, where angular displacement of the fragments during the process of union did occur, the cartilage took a definite shape, namely, wedge or bracket shape, with the point toward the space between the ends of the cortex of the fractured bone and the base outward against the periosteum. The width of this bracket was in direct proportion to the amount of angular displacement of the fragments. The amount of periosteal callus on the internal side of the angle was also increased in direct proportion to the amount of displacement. It was proportionately decreased on the opposite side, in some instances being very small in amount (Figs. 6 and 7). Sufficient osteoid tissue is formed on the internal aspect of the angle to again reëstablish the general line of the bone from one point to the other in order to stand the strain required to perform the function of the limb. The

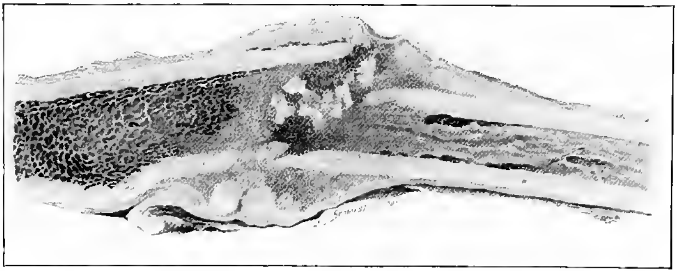

Fig. 5.—Experiment 20, Series 2, fracture thirty-five days. A drawing illustrating a fracture with slight lateral displacement. The cartilage is present in the form of islands across the line of fracture, and not as a wedge.

denser portions of the new trabeculæ are on the most external portions of the callus, extending as a solid line from the old cortex to the base of the wedge of cartilage. In the deeper portions the callus is much more cancellous in appearance. Microscopically, this is seen to be caused by great osteoclastic absorption of the deeper layers of the trabeculæ in lines parallel to the line of the original bone (Fig. 8). There is considerable absorption of the cortex and the medullary trabeculæ.

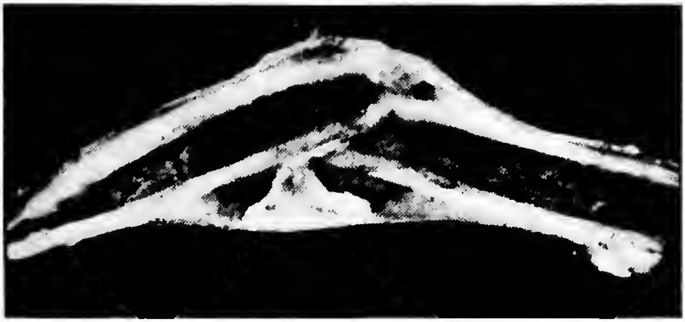

FIG. 6.—Experiment 21, Series 2, fracture fifty-eight days Illustrates a fracture with considerable angular displacement having abundant callus with a large wedge of cartilage on the internal aspect of the angle.

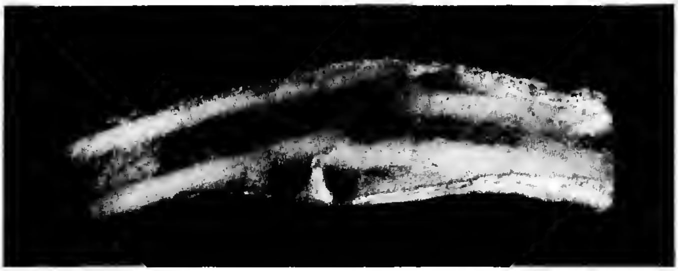

FIG. 7.—Experiment 22, Series 2, fracture sixty-three days. Illustrates a fracture with but slight angular displacement having a small wedge of cartilage on the periosteal aspect of the callus.

The tendons acted as a barrier to the formation of callus, forming grooves from which they had to be dug to release them.

Microscopically, osteoid trabeculæ do not appear between the cortical ends of the fragments until the eighty-fifth day, and then in very small amount only, continuous with the medullary trabeculæ. Islands of cartilage are intermingled with them across the line of fracture. In a fracture of one hundred and twenty-nine days, in which there was very good position of the fragments, there is an entire absence of cartilage with great absorption of all the trabeculæ, the large spaces being again lined with a new deposit of bone which

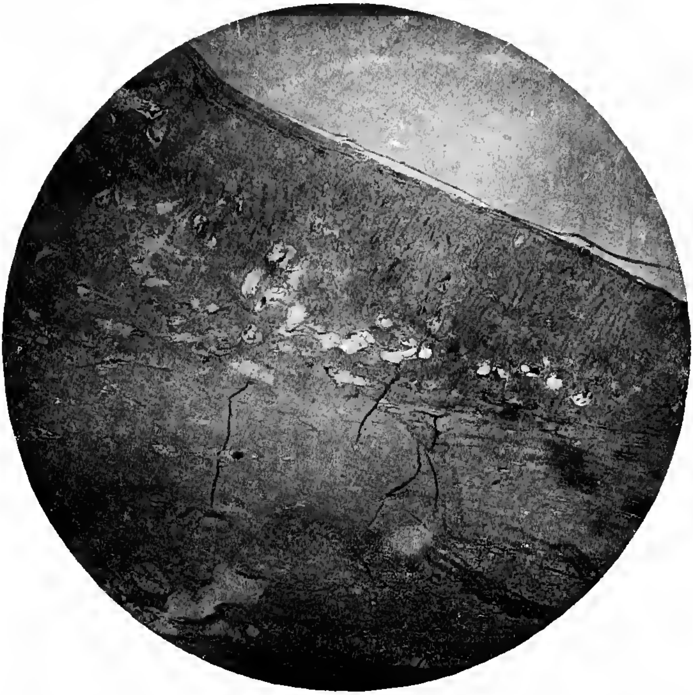

Fig. 8.—Experiment 21, Series 2, fracture fifty-seven days. Demonstrates the absorption that occurs in the deeper layers of the periosteal callus with the formation of spaces parallel to the cortex This is part of the rearrangement or reorganization that occurs to accommodate for new conditions of stress and strain.

resembles the lamellæ of the old cortical bone, the cells being more spindle in shape than in ordinary osteoid tissue and the bone appearing denser in nature (Fig. 9). That new deposit is probably true osseous tissue. There are osteoid trabeculæ in between the cortical ends of the fragments which exhibit great absorption; also a deposit of new bone and union of the Haversian spaces of the old cortical ends. The union of the fracture is approaching completion, but the new tissue is still very porous. In another specimen, also, two hundred and sixty-two days in duration, in which there was good apposition of the fragments, there is osteoid

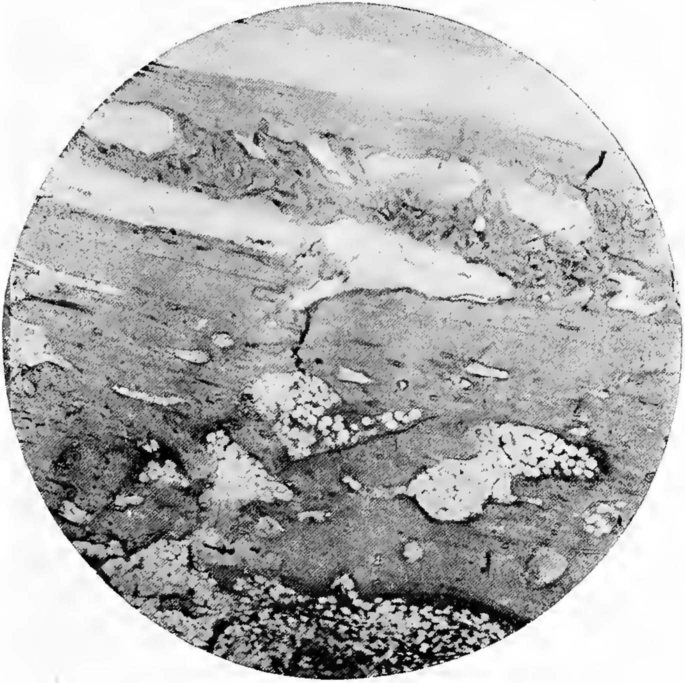

FIG 9.—Experiment 25, Series 2, fracture one hundred and twenty-nine days. Illustrates the extensive absorption that occurs in the cortex and osteoid trabeculæ, followed by a deposition of new osseous tissue in the widened spaces. The new bone is seen as a very thin rim lining the dilated spaces (upper portion of figure).

tissue between the ends of the cortical bone, it being denser, with more numerous and smaller spaces than the old cortical bone, which shows absorption. Its spaces communicate with the Haversian spaces of the old cortex. In this specimen the displacement was slightly greater than in the last one and there was, conseqeuntly, more cartilage present, the remnants of which are found in the periosteal callus as a very small wedge. Both the external and internal callus have undergone great absorption. In another specimen, two hundred and seventy-seven days in duration, there is still remaining a thin line of fibrocartilage at the site of the fracture, but not in the shape of a wedge. It is undergoing transformation into osteoid trabeculæ.

In this series the transition of cartilage into osteoid trabeculæ appears to occur in two different ways. It does not occur in the body of the mass of cartilage, as in the earlier stages of the development of callus (see Rabbit Experiments), but occurs only at the edge of the wedge (Fig. 10). Here the process appears to have a definite relation to the large osteoclasts which, in some cases, appear to invade the cartilage, absorb the ground substance, liberate the cells, and make way for the invasion of bloodvessels. This is to be followed by a deposition of lime salts on the borders of the cavities created, as in embryonal endochondral bone formation. In other areas, the deposit of lime salts appears to precede and then there is an invasion by bloodvessels and the formation of medullary spaces.

This completes the two series in which complete subcutaneous fractures were studied with the object of investigating the subject as found clinically and with the object of confirming or denying the findings of other writers on the subject, particularly Dupuytren. Experiments performed in this manner, however, are unsatisfactory, for no two cases are under exactly similar conditions with reference to mobility, fragmentation, line of fracture, displacement, pressure from bandage, etc. There is no method of isolating the actually active portions of the bone and determining whether there is a definite reciprocal relation between the activities of the different factors of the bone. Each portion has been given credit for the chief or sole activity in the repair of fractures, and probably with much reason, because of the varying conditions of the specimens. In my own experiments as above described, while the general progression of the repair was more or less uniform, the comparative amounts of tissue from one or other portion of the bone varied in the different specimens. I think that experiments on rabbits are particularly unsatisfactory owing to the great difficulty in obtaining and maintaining good position of the fragments.

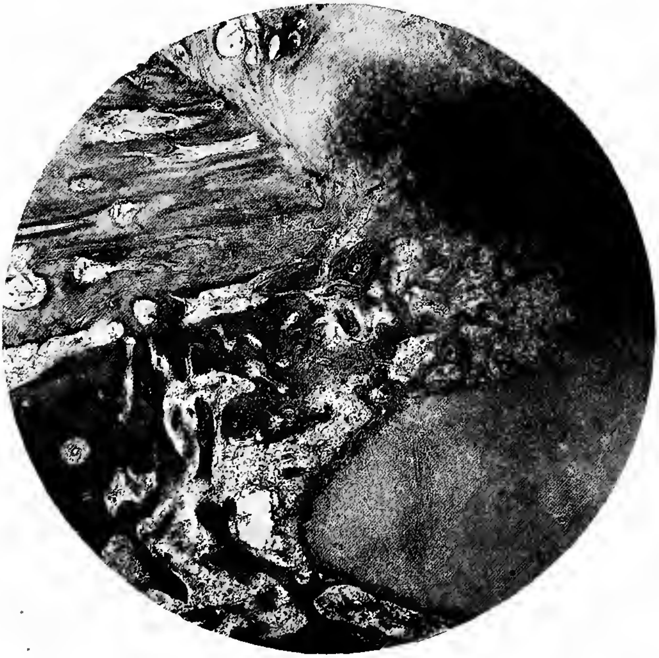

Fig. 10.—Experiment 21, Series 2, fracture fifty-seven days. Illustrates the transition of cartilage into osteoid tissue that occurs at the edge of the wedge of cartilage.

Having arrived at the above conclusion, the other series of experiments to be described were next carried out.

SERIES 3.

In this series the animals were placed under the influence of an anesthetic, an incision made over the inner aspect of the tibia down to the bone, and then a saw cut made in the bone until it penetrated the medullary cavity. In this manner all portions of the bone were cut across and injured, but there were no extrinsic forces at work to influence union. The wound was immediately closed with silk sutures and the leg placed in a shaped wooden splint with a soda silicate bandage around it. The animals were killed at varying intervals from five to seventy days. Altogether, fourteen successful operations free from suppuration, etc., were obtained.

The specimen obtained at the end of five days exhibits no proliferation of the periosteum whatever, but in the medullary cavity there is an increase of the connective tissue between the fat cells which appears to obliterate them, for on the outskirts there can be noticed a gradual increase of the loose connective tissue with a commensurate decrease in the number of fat cells. This new tissue is myxomatous in nature, many of the cells being typically stellate, others spindle with long, filamentous ends (Fig. 11). The substance between the cells is not very distinct, so that it is difficult to say whether this tissue is true myxomatous tissue or merely very loose connective tissue The main point in favor of it being myxomatous in character, if not true myxoma, is the fact that on gross examination this tissue always resembles cartilage and has a glistening appearance. It was also quite firm. The new trabeculæ follow in the line of this new tissue. On the inner aspect of the cortex there is distinct thickening of the medullary membrane, with the formation of large polygonal cells which, in areas, appear to be secreting a homogeneous substance which takes the eosin staining. This matrix becomes more abundant, the cells rearrange themselves in layers on its surface (some few being caught and imprisoned in it), and we have the formation of osteoid trabeculæ. Similar trabeculæ are forming on the spicule of bone that had been driven into the medullary canal by the saw. The sawcut is filled with blood clot and contains no formative cells.

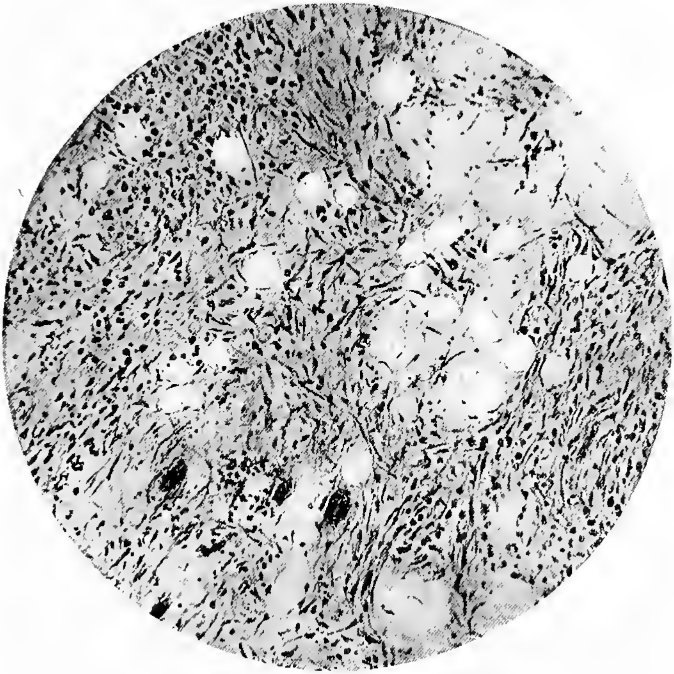

Fig. 11 —Experiment 44, Series 4, fracture nine days Shows the filamentous myxomatous character of the connective-tissue proliferation that occurs between the fat cells of the medulla.

At the end of seven days beginning signs of activity in the periosteum can be seen at some distance from the site of operation, there being proliferation of the deeper layers of the periosteum, with the production of the large formative cells in the osteogenetic layer. What appears to be more worthy of note is the fact that the periosteal surface of the cortex shows absorption by large polynuclear cells which are forming pits on the surface of the bone by the absorption of the lime salts. This, I believe, has considerable bearing on the future development of the periosteal osteoid trabeculæ. In the medullary cavity the process described under the last experiment is present to a greater degree, the formation of trabeculæ extending half-way across the medullary cavity and bridging over the wound in the bone. There is also a hyperplastic increase in the marrow, with the formation of red cellular marrow on the outskirts. With the exception of accentuation of the processes there is little change from the seventh to the ninth day, when the osteoid tissue in the medulla enters the sawcut and fills it about half the distance to the surface, the remainder being filled by fibroblasts and young bloodvessels with fibrin most superficial. The cortex proper, as well as its periosteal surface, begins to exhibit signs of slight absorption. There are no trabeculæ in the periosteum and but slight proliferation except where there is superficial absorption of the cortex.

On the tenth day there is the first evidence of osteoid trabeculæ in the periosteum. In this particular specimen, in which there is more than the usual amount of inflammatory reaction, there is a fair amount of periosteal bone formation everywhere except at the margins of the sawcut, where it is absent. The sawcut is filled in with granulations into which osteoid trabeculæ are growing from the medullary trabeculæ (Fig. 12). In the medullary cavity there are osteoid trabeculæ surrounded by connective-tissue hyperplasia of the marrow, in which osteoid trabeculæ can be seen growing. This hyperplasia progresses from the edges of the new formation and occurs around groups of fat cells and not around individual cells, so that a scaffolding of connective-tissue trabeculæ is formed, upon which is constructed the trabeculæ formation (Fig. 13). The actual deposit of calcareous salts always begins from the cortex inward, that is, in contact with previous calcareous tissue. This specimen demonstrates clearly the method of trabeculæ formation from the periosteum, as described under the rabbit experiments, showing the two layers of the periosteum and the formation of the trabeculæ from the deeper layer. The trabeculæ nearest the cortex are thicker and the spaces between them smaller, proving that they had been formed first. There is increasing absorption of the cortex.

From the tenth to the seventeenth day little change is to be noted, there being no new developments, but merely an increase in all the elements noted above, viz., the periosteal, medullary, and sawcut trabeculæ. *All the trabeculæ in the sawcut are medullary in origin and course in a direction at right angles to the long axis of the bone.* They do not appear to have any attachment to the side of the bone until absorption of the sides of the sawcut occurs. The nutrient artery, instead of assisting in the formation of callus, can act as a barrier. In one specimen the trabeculæ follow along the

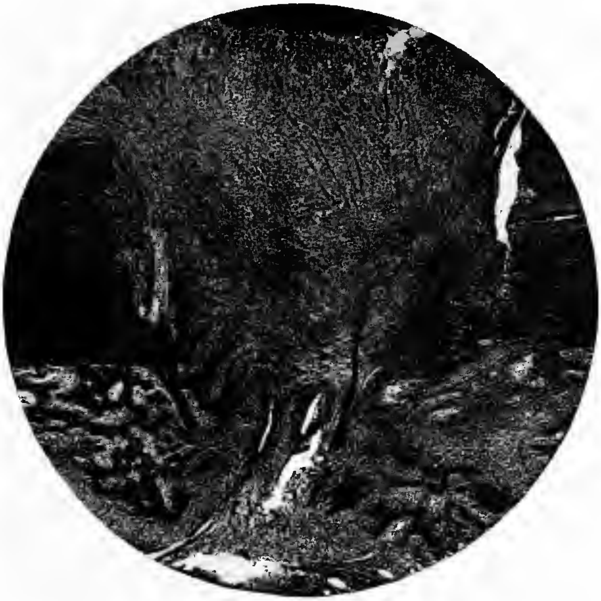

FIG. 12.—Experiment 32, Series 3, fracture ten days. Shows osteoid trabeculæ growing into the sawcut from the medulla (below), also absorption of the cortex along the sides of the cut.

artery for its entire length on the side of the fracture and are entirely absent on the other side.

As the periosteal trabeculæ increase, the general trend of the trabeculæ and of the spaces between the trabeculæ becomes very oblique, gradually becoming more and more in the general line of the bone. As early as the seventeenth day there is a certain amount of absorption of the deeper trabeculæ in a line with the old cortex, forming a space that runs almost parallel with the old cortex and just superficial to it, giving channels of communications with the spaces in the somewhat rarefied old cortex (Fig. 14).

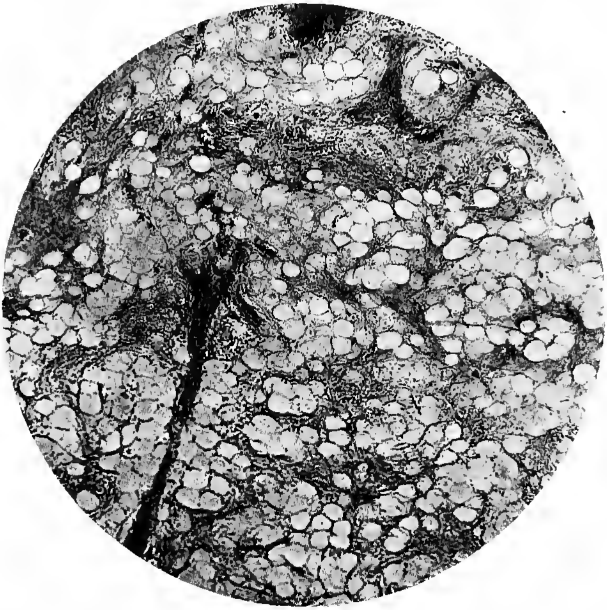

FIG. 13.—Experiment 35, Series 3, fracture fifteen days. Demonstrates the formation of skeleton connective-tissue trabeculæ in the medulla by proliferation around and between groups of fat cells. It also illustrates how the osteoid trabeculæ grow into and along these trabeculæ of connective tissue (top).

The method of progression of the osteoid trabeculæ in the sawcut from the medulla to the surface is about as follows:

The osteoblasts proliferate, elaborate a matrix, or cause modification in the tissue in which they lie. This is followed by a deposit of lime salts and the formation of the trabeculæ. These trabeculæ are covered by osteoblasts, some of which are in process of inclusion, being half in and half out of the osteoid mass (Fig. 15). From the seventeenth day absorption of the periosteal and endosteal trabeculæ occurs, but is more marked in the medullary tissue. Here great numbers of large multinuclear cells with a large amount of protoplasm (Fig. 16) are to be found in relation with the trabeculæ,

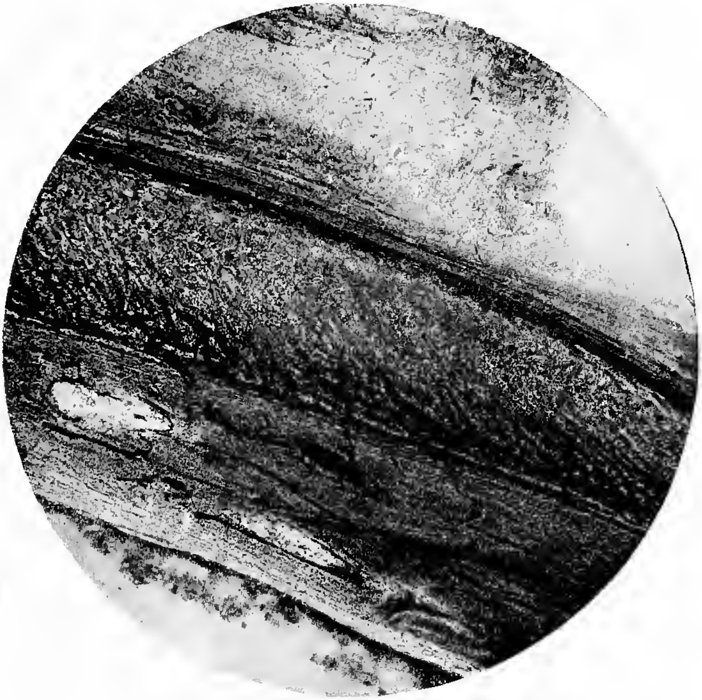

FIG. 14.—Experiment 37, Series 3; fracture seventeen days Ground specimen. Shows the presence of a large amount of periosteal osteoid trabeculæ (above), the spaces between which communicate with the Haversian canals of the cortex. One long space runs superficial to and parallel with the cortex. The intertrabecular spaces are quite oblique in direction.

which show pits and depressions everywhere. Examination of the process under high powers demonstrates how the osteoclast opposes itself to the side of the trabeculæ and apparently dissolves out the calcareous salts and liberates the cells contained in them. These cells are not dead, but, as soon as released, the protoplasm again becomes evident,

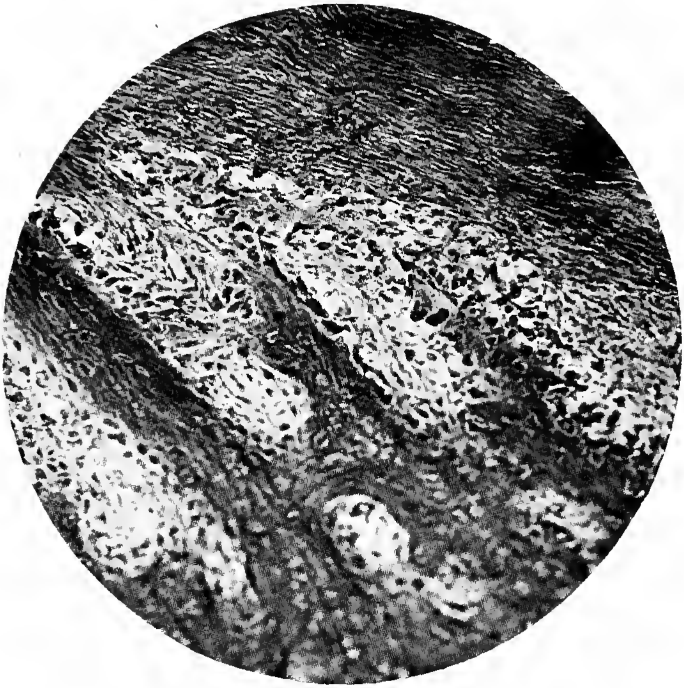

FIG. 15.—Experiment 38, Series 3, fracture nineteen days Demonstrates the method of formation of the sawcut trabeculæ, the osteoblasts being on the surface of the older trabeculæ, while they are in the process of inclusion in the younger forming trabeculæ, some of the cells being half within and half without the homogeneous osteoid mass

and the cell assumes a rounded shape, with a well-staining nucleus and a small amount of protoplasm, resembling in many respects the normal marrow cell. When liberated they do not look at all like the osteoblasts from which they were formed, but more like the small marrow cells from which, possibly, the original osteoblast dates its origin.

About this time absorption of the cortical sides of the sawcut, also, proceeds rapidly with the invasion of the trabeculæ in the sawcut and the establishment of communications between the spaces in the cortical bone and those between the trabeculæ in the sawcut. This is found in its earlier stages, when the general trend of the trabeculæ is still at right angles to the general line of the bone (Fig. 17). On the thirty-second day the osteoblasts have practically

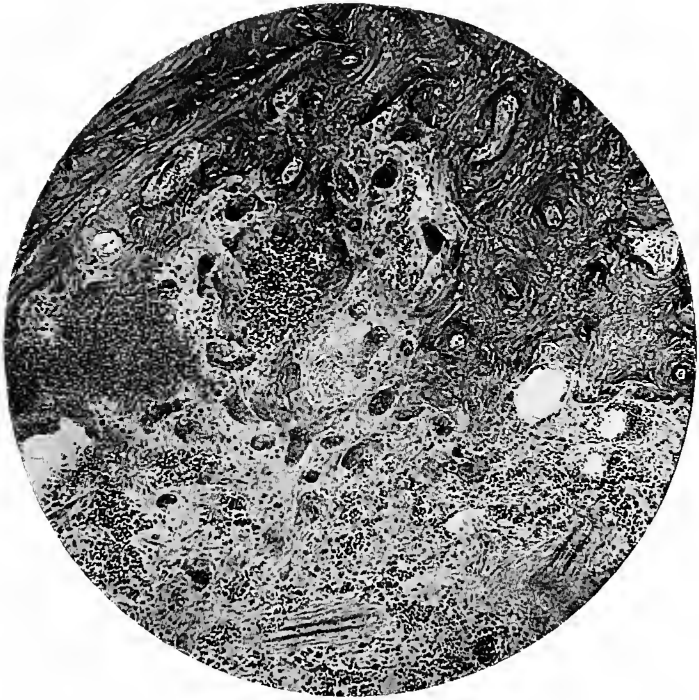

Fig. 16.—Experiment 39, Series 3, fracture twenty-two days. Shows a large number of multinuclear osteoblasts which are causing extensive absorption of the medullary osteoid tissue. It also shows the cellular character of the marrow

disappeared from the periosteal and medullary trabeculæ; demonstrating that their activity has practically ceased. There are no specimens in this series between the thirty-second and seventieth day, at which time a considerable change is noticed.

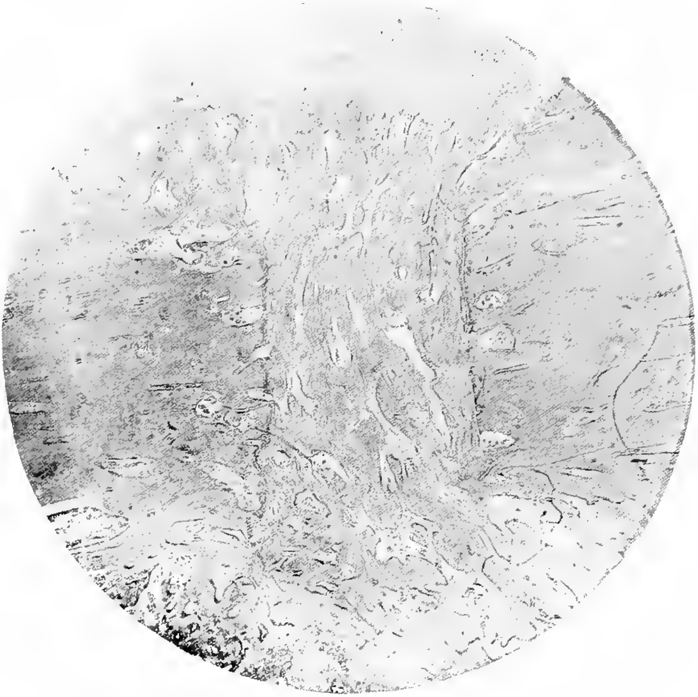

FIG. 17.—Experiment 39, Series 3, fracture twenty-two days Demonstrates the vertical trend of the trabeculæ in the sawcut, absorption of the cortex on the sides of the cut and beginning communications between the new and old Haversian spaces.

On the periosteal aspect of the seventy-day fracture there is a very compact, rather thick layer of new bone, which is continuous with the bone in the sawcut without a sign of a depression or break. Its outer layer exhibits considerable lacunar absorption. Here, as shown in Fig. 18, which is a wash drawing from a high power, photography being unsatisfactory, the process is practically similar to that which occurs in the medullary cavity. The osteoclast appears to dissolve out the lime salts and liberate the bone cells. What their fate is in this instance cannot be stated, as they cannot be found very far from the surface of the bone. They first appear as small round cells, with a moderate amount of protoplasm and well-staining nuclei. As they get farther away from the side of the bone they seem to get smaller and finally disappear. They probably degenerate, but of this I cannot be positive.

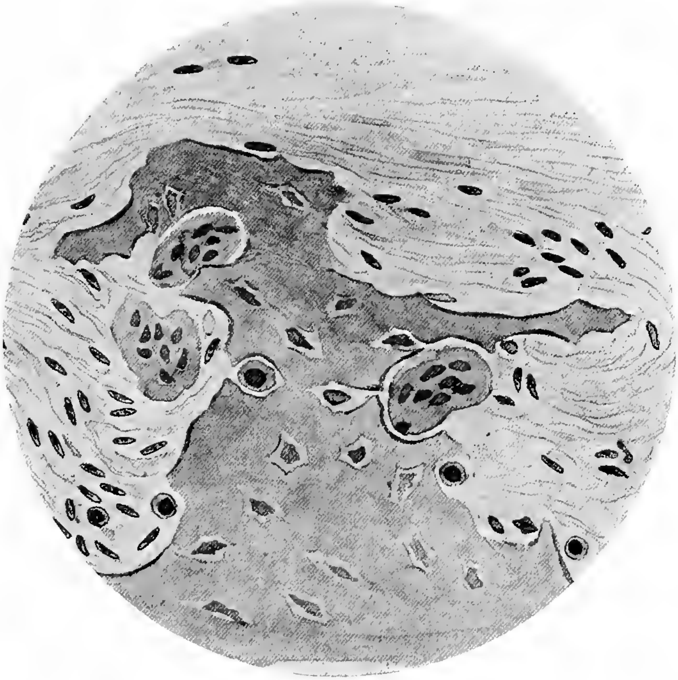

FIG. 18.—Experiment 41, Series 3, fracture seventy days. Wash drawing showing how the osteoclasts dissolve the calcium salts in the periosteal trabeculæ and free the bone cells which become lost in the connective tissue.

CHART 3.

Number.	Series.	Days.	Trabeculæ in			Osteoblasts on			Absorption of					Marrow.	Direction of medullary spaces between sawcut trabeculæ with reference to the axis of the bone.	Communications between old Haversian canals of cortex and those between sawcut trabeculæ.
			Periosteum.	Sawcut.	Medullary cavity.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.					
											Sawcut	Interstitial.	Surface.			
28	3	5	—	—	+	A	+	+	A	—	—	—	—	Pink.	A	A
29	3	7	—	—	+	A	+	—	A	—	—	—	+	Red.	A	A
30	3	8	—	—	+	A	+	—	A	—	—	—	+	Red.	A	A
31	3	9	—	½	+	A	+	—	..	—	—	Sl.	+	Red.	L	—
32	3	10	+	Few.	+	+	+	—	—	—	+	+	+	Red.	L	—
33	3	11	+	+	+	+	+	—	—	?	—	+	—	Red.	L	—
34	3	12	+	Few.	+	+	+	—	—	—	+	+	+	Red.	L	—
35	3	15	+	+	+	+	+	—	—	—	—	+	+	Red.	L	—
36	3	16	Sl.	+	+	+	+	+	—	—	+	+	+	Red.	L	—
37	3	17	+	+	+	+	+	+	?	+	+	+	+	Red.	L	—
38	3	19	+	+	+	+	+	—	—	—	—	+	—	A	L	—
39	3	22	+	+	+	+	+	—	—	+	+	+	Sl.	Red.	L	+
40	3	32	+	+	+	—	—	—	+	+	—	+	—	Red.	L	+
41	3	70	+	+	+	—	—	—	+	+	+	+	Sl.	Red.	=	+

Explanation. + present. — absent. A, primary tissue absent. Sl., slight. ½, half-way across. L, at right angles. = parallel. ? doubtful.

Chart 4.

Number.	Series.	Days.	Trabeculæ in			Osteoblasts on			Absorption of					Marrow.	Direction of medullary spaces between sawcut trabeculæ with reference to the axis of the bone.	Communications between old Haversian canals of cortex and those between sawcut trabeculæ.
			Periosteum.	Sawcut.	Medullary cavity.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.					
											Sawcut	Interstitial.	Surface.			
42	4	5	A	Few.	Few.	A	+	—	A	—	—	+?	—	Red.	L	—
43	4	7	A	Few.	Few.	A	+	—	A	—	+	+	—	Red.	L	—
44	4	9	A	+	+	A	+	?	A	—	—	—	—	Red.	?	?
45	4	12	A Few	+	+	+	+	+	—	+	+	+	+	Red.	L	+
46	4	15	A	+	+	A	+	—	A	+	+	+	+	Pink.	L	Beginning.
47	4	19	A+	+	+	+	+	—	—	+	—	+	+	Red.	L	—
48	4	23	A	+	+	A	+	+	A	+	+	+	+	Red.	L	+
49	4	28	A+	+	++	+	+	+	+	+	+	+	+	Red.	L	+
50	4	49	A	+	+	A	—	+	A	+	+	+	+	Pink.	=	+

Explanation: + present. — absent. A, primary tissue absent. ? doubtful. L, at right angles. = parallel.

The sawcut is very interesting and instructive. It is entirely filled with more or less compact bone in which the trend of the medullary spaces between the trabeculæ has

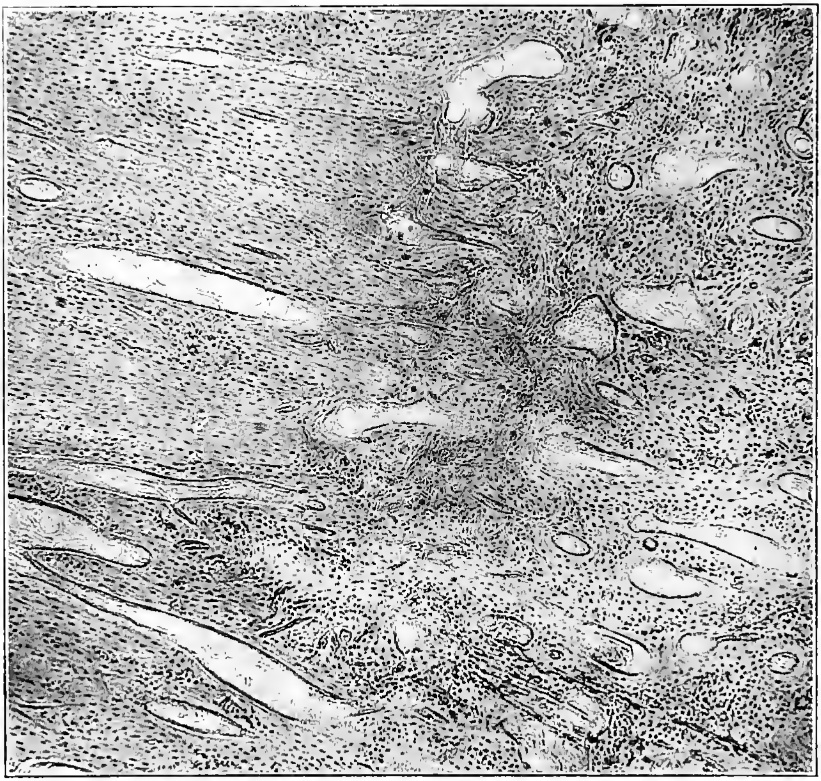

FIG. 19.—Experiment 41, Series 3, fracture seventy days. Demonstrates how the spaces between the trabeculæ in the sawcut change from a vertical to a horizontal direction parallel with the line of the bone, and how they unite with the Haversian spaces of the cortex, this being followed by a deposit of new bone in the two. Three stages of bone formation can be seen. Old cortex to left and sawcut to right of picture.

changed from at right angle to the line of the bone to a direction parallel with it (Fig. 19). Differences in staining show how the old vertical spaces had been filled in and new transverse spaces made which originally were very large. These new spaces communicated with similar spaces in the old cortex. This was followed again by a deposition of new bone which was continuous throughout the new space, forming a firm bond of union between the old cortex and the new trabeculæ. The new deposit is in the form of lamellæ and the cells in them more of the normal spindle shape of bone cells. Thus we see that under conditions where no extrinsic forces have been at work the process of repair is practically complete at the end of seventy days, showing further progress than the complete fractures did at the end of two hundred and sixty-two days. In addition, there is not the slightest suggestion of cartilage in any of the specimens.

SERIES 4.

While Series 3 isolated to a greater or less extent the activities of the different portions of the bone and demonstrated their various activities, the other series were performed to ascertain whether the activities of one portion could be substituted for those of another. In Series 4 the action of the medulla and cortex, without the assistance or influence of the periosteum, was studied. The animals were placed under the influence of an anesthetic and an incision made over the inner aspect of the tibia. The bone was denuded of periosteum for about one to one and a half inches in the region of the operation, and the periosteum cut off. A sawcut was then made in the bone sufficiently deep to enter the medullary cavity. The wound was sutured and a splint applied with a sodium silicate bandage.

The results in this series correspond very closely to those of the previous series with reference to the activities in the bone and medulla, with the exception that these seem to be somewhat increased. The absorption that occurs in the cortex begins early and is more marked than in the case where the periosteum was intact. The sawcut is filled in the same manner as (and, if anything, slightly sooner than) in the last experiment, since by the twelfth day it is completely filled in and "overflows" the edge of the cut, appearing to a small extent on the periosteal aspect of the cortex. The communications between the dilated cortical spaces and the spaces between the trabeculæ in the sawcut are established much sooner, being present as early as the twelfth day. The medullary trabeculæ fill the sawcut so rapidly that by the nineteenth day they extend well over the periosteal surface of the cortex and "overflow" upon it (Fig. 20). They also fill in the normal dilated cancellous spaces in the cortex that have been cut across.

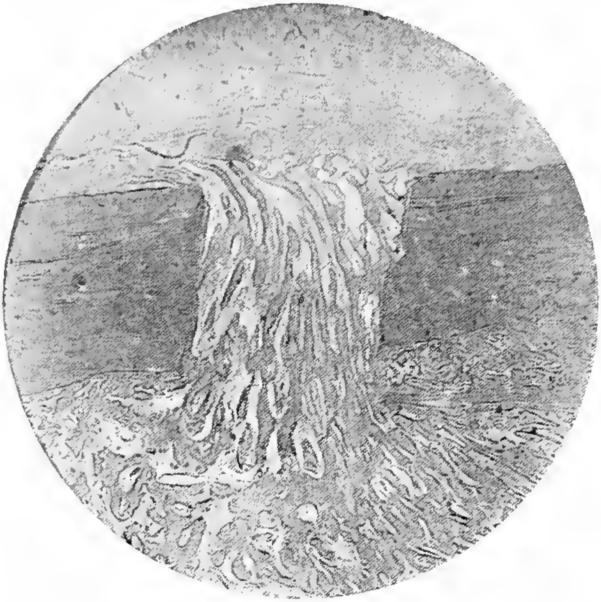

Fig. 20.—Experiment 47, Series 4, fracture nineteen days. Illustrates the sawcut filled with medullary trabeculæ "overflowing" the periosteal side (above) of the cortex where the periosteum had been denuded. They also fill a normal cancellous space in the cortex (right).

Absorption of the cortex proceeds rapidly and is extensive in the region of the sawcut (Fig. 21) in marked contrast to the remainder of the bone.

The healing of the defect progresses so rapidly that the absorption of the medullary trabeculæ begins as early as the twelfth day, whereas in the previous series it did not begin until the seventeenth day. Absorption of the surface of the cortex is not found until the twelfth day, but it continues throughout the remainder of the experiments, until the forty-ninth day, in contrast with the previous series, in which it almost ceases at the end of the seventeenth day. Osteoblasts disappear from the surface of the medullary trabeculæ by the forty-ninth day, but are present in the cortex from the twenty-third to the forty-ninth day.

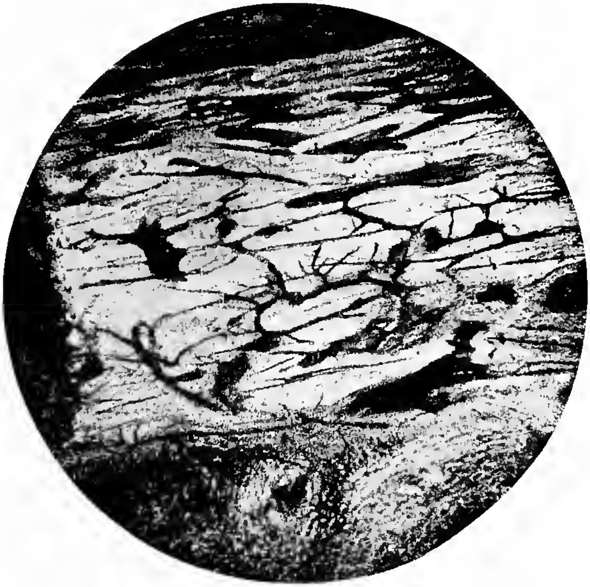

Fig. 21.—Experiment 48, Series 4, fracture twenty-three days. Ground specimen. Shows extensive absorption of the cortex in the region of the sawcut (left) with a tendency for these dilated spaces to unite with the spaces between the medullary osteoid trabeculæ (below).

The specimen from a forty-nine days' fracture shows the first stages in the change of direction in the medullary spaces between the sawcut trabeculæ. As in the last experiment of the previous series, the old spaces are being filled in and new spaces, lined with osteoblasts, are being formed, communicating with similar spaces in the old cortex (Fig. 22). The marrow, in all the experiments of the series, is hyperplastic with an increase in cellular content. In almost all of the specimens the periosteum, where it was still attached at the point of excision, shows a liberal amount of new bone. On the opposite side of the bone there is lacunar absorption and a proliferation of the osteoblastic layer of the periosteum without the formation of bone

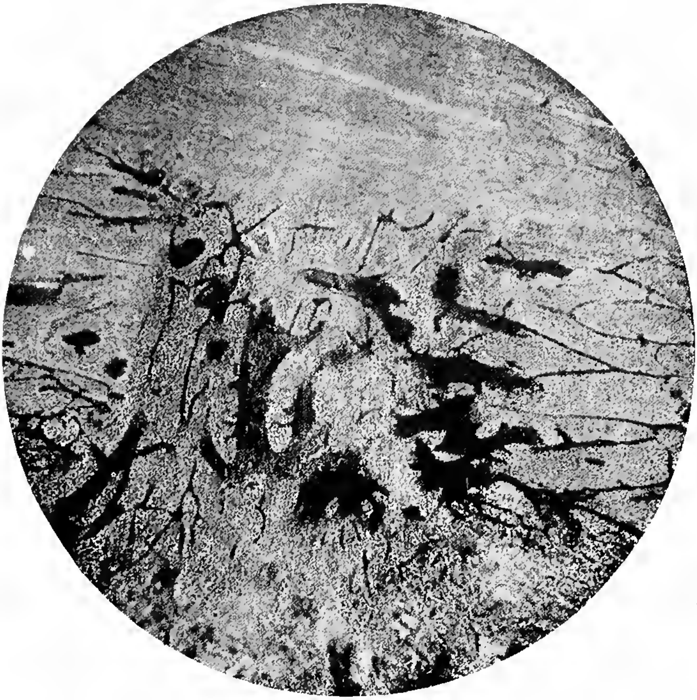

Fig. 22.—Experiment 50, Series 4, fracture forty-nine days. Ground specimen. Shows extensive absorption of the sides of the sawcut creating spaces which unite the Haversian canals of the cortex with the spaces between the trabeculæ in the sawcut.

SERIES 5.

The method of operating for the performance of this series of experiments was identical with that for Series 4, with the exception that the sawcut was superficial and did not enter the medullary canal. Thus, with the periosteum removed and the medulla not disturbed the activity of the cortex could be determined. Eight successful specimens have been obtained. The results, in brief, of this series are as follows:

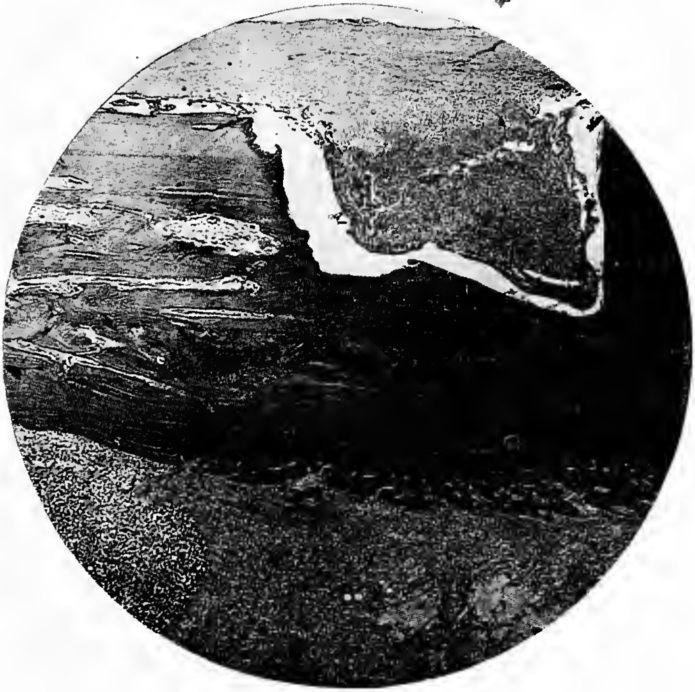

Fig. 23.—Experiment 54, Series 5, fracture twenty-four days Shows a shallow sawcut filled with proliferated connective tissue and blood clot, moderate absorption of the cortex and a moderate amount fo osteoid tissue on the medullary aspect of the cortex (below).

Absorption of the cortex proper is the most constant result. It is present to a very marked degree in all the specimens and increased in quantity, depending on the depth of the sawcut. In only one specimen (a fracture of twelve days' duration) were there any periosteal trabeculæ in the region of the wound. It is probable that in this case the entire periosteum was not removed, but merely the fibrous layer. In almost all of the specimens the medulla shows

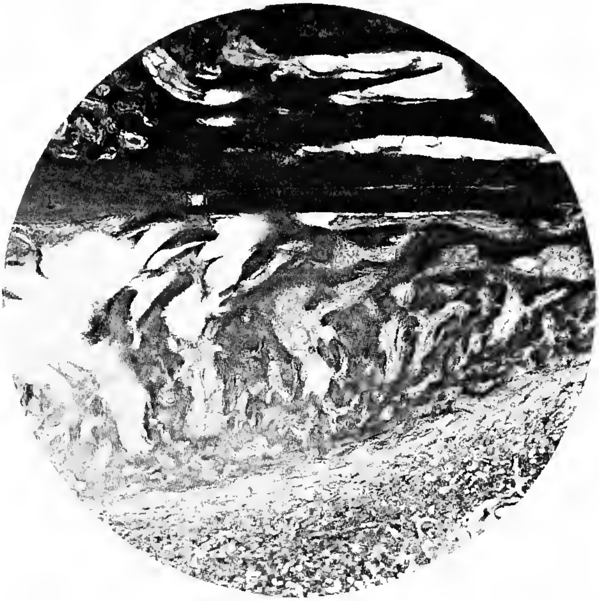

Fig. 24.—Experiment 53, Series 5, fracture twelve days. Illustrates the large amount of medullary osteoid tissue that is associated with a deep sawcut (upper left-hand corner) and absorption of the cortex. The medullary trabeculæ are limited by a membrane that resembles the deeper layer of the periosteum.

signs of irritation in the increased cellular content of the marrow. In four of the eight specimens there is a growth of osteoid tissue in the medullary cavity. The extent of this growth depends more on the depth of the sawcut and the extent to which absorption of the cortex had progressed than upon the duration of the fracture (Figs. 23 and 24).

CHART 5.

Number.	Series.	Days.	Trabeculæ in			Osteoblasts on			Absorption of					Marrow.	Direction of medullary spaces between the sawcut trabeculæ with reference to the axis of the bone.	Communications between old Haversian canals of cortex and those between sawcut trabeculæ.
											Cortex.					
			Periosteum.	Sawcut.	Medullary cavity.	Periosteal trabeculæ.	Medullary trabeculæ.	Cortex.	Periosteal trabeculæ.	Medullary trabeculæ.	Sawcut	Interstitial.	Surface.			
51	5	3	A	—	—	A	—	+	A	A	—	+	—	Sl. con.	A	A
52	5	5	A	—	—	A	A	—	A	A	—	?	—	A	A	A
53	5	12	A+	+	+	+	+	—	—	—	+	+	—	Pink.	Indifferent.	+
54	5	24	A	—	+	A	A	+	A	A	—	+	—	Red.	A	A
55	5	30	A	+	—	A	?	?	A	—	+	+	—	Red.	— ?	— ?
Ground.																
56	5	35	A	—	—	A	A	+	A	A	+	+	?	Red.	A	A
57	5	45	A	+	+	A	?	?	A	?	—	+	?	Red.	=	+
Ground.																
58	5	70	A	+	+	A	—	—	A	+	+	+	+	Red.	Indifferent.	+

Explanation: + present. — absent. A, primary tissue absent. ? doubtful. Sl. con., slight congestion. = parallel.

This growth occurs as a smooth, well-defined elevation, from the inner aspect of the cortex, unaccompanied by considerable hyperplastic increase of connective tissue in the marrow, but, especially in one specimen (Fig. 24), by the condensation of the tissue in the region of the growth with the formation of a membrane almost similar to the deeper layer of the periosteum. *In none of these specimens is there a diffuse increase of connective tissue in the marrow, with the formation of the skeletal trabeculæ, and in none of the experiments is there an irregular deposit of osteoid trabeculæ.*

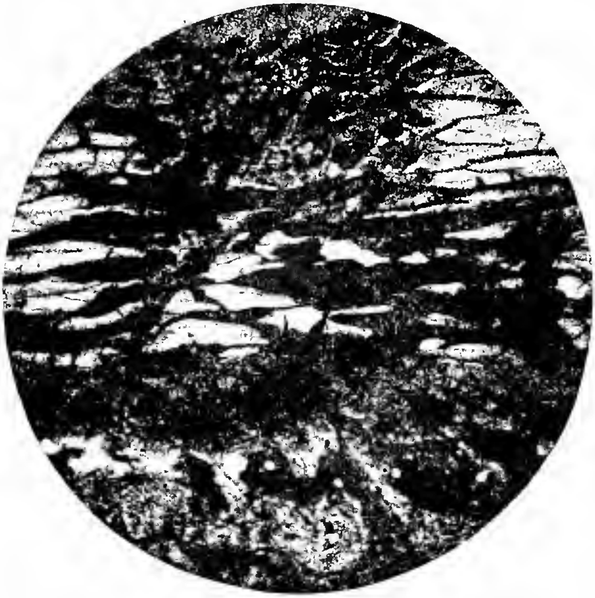

Fig. 25.—Experiment 57, Series 5, fracture forty-five days. Ground specimen. Illustrates the extensive absorption of the cortex that occurs near a shallow sawcut, especially between it and the medullary cavity.

In four of the experiments the sawcut contains osteoid tissue. It appears first on the twelfth day, as a small amount of thin trabeculæ at the base of the cut, showing no general direction to the spaces, which are large and rounded. There is a great amount of absorption of the cortex and a large mass of trabeculæ in the medullary canal.

A ground specimen from a fracture of thirty days also exhibits a similar condition, but in this case there is not so much absorption of the cortex. A specimen from a thirty-five-day fracture does not contain trabeculæ in the sawcut, but in this case the injury was very shallow and the development of all the stages of the process has been very slow. There is a small amount of osteoid tissue in the medullary cavity, and also absorption.

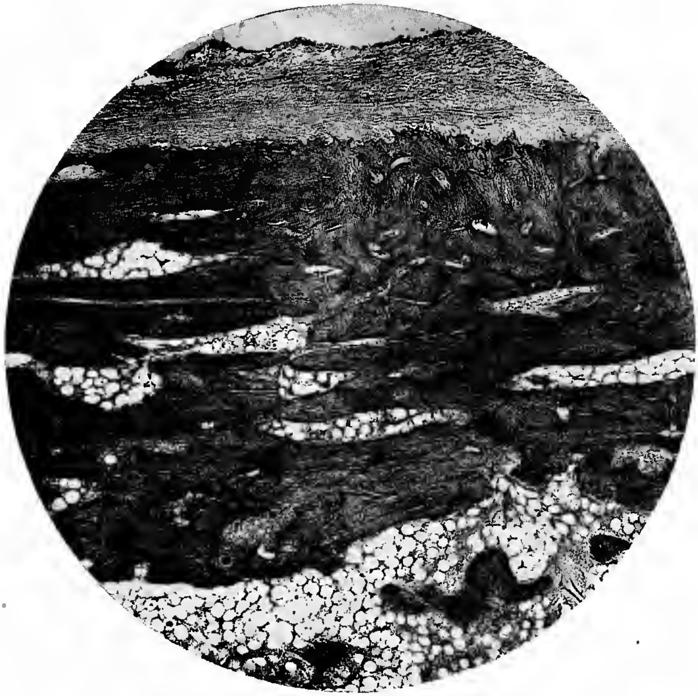

Fig. 26.—Experiment 58, Series 5, fracture seventy days. The sawcut (above) is filled with new compact bone, which is undergoing lacunar absorption upon its surface. There is marked absorption of the cortex with a tendency to the formation of communications between the cortical and new bone spaces. There is some new bone formation in the medullary cavity.

A specimen from quite a deep fracture of forty-five days' duration shows marked response in all portions of the bone. There is considerable absorption of the cortex, with a large amount of osteoid trabeculæ in the medullary cavity and in the sawcut (Fig. 25). A similar condition is found in a fracture of seventy days' duration (Fig. 26), with the exception that most of the medullary trabeculæ are absorbed and the surface of the trabeculæ in the sawcut is undergoing lacunar absorption (Fig. 27). In both of these specimens there is no definite direction to the spaces between the trabeculæ, but the general trend is more in the line of the bone than transversely. Throughout this series there is practically no absorption of the periosteal surface of the cortex.

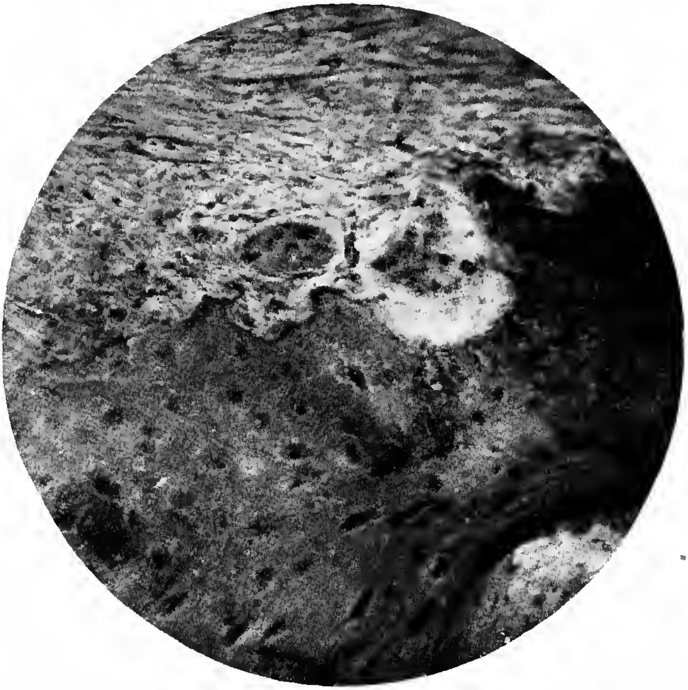

Fig. 27.—Experiment 58, Series 5, fracture seventy days. A high-power photomicrograph of the lacunar osteoclastic absorption seen upon the surface of the last specimen. It shows the liberation of bone cells.

SERIES 6.

This series, performed upon dogs, had as its object the determination of the activity of the periosteum when raised from the bone, associated with injury to the bone itself, not deep enough to enter the medullary canal. Each operation consisted in exposing the inner side of the tibia, raising the periosteum, and, in some instances, cutting one end of the raised periosteum, and, in other instances, raising the periosteum without cutting it transversely. The bone was then injured superficially with rongeur forceps or shallow sawcuts. Where the periosteum had been cut transversely it was sutured in place with catgut, and where this transverse section had not been made it was merely allowed to fall back into place The wound was then sutured and covered with collodion. Ten successful specimens were obtained, the duration of the injuries ranging from two to forty-nine days. The results in this series, while not perfectly uniform, all tend in the same direction. After the eighth day they exhibit proliferation of the periosteum and bone formation, with the exception of the cases in which the raised periosteum had not again adhered to the cortex. Here the periosteum is proliferated with an apparent effort at trabecular formation, but there is no deposit of calcium salts in them. The proliferated periosteum dips into the sawcuts and in some of the specimens has apparently proceeded to form new bone. Whether this new bone in the base of the sawcut is always derived from the periosteum or from the medullary tissue in the cortex, as in the experiments of Series 5, it is impossible to state, for there are no trabeculæ in the sawcuts where there is no absorption of the cortex and lining of osteoblasts in its dilated spaces. The only apparent exception to this is Experiment 68, forty-nine days in duration. Here the process was probably already complete and the spaces filled in.

CHART 6.

Number.	Series.	Days.	Proliferation of deeper layers of raised periosteum.	Osteoid trabeculæ in the raised periosteum.	Trabeculæ in medullary cavity.	Marrow.	Absorption of cortex.			Osteoblasts in cortex.
							Sawcut.	Interstitial.	Surface.	
59	6	2	—	—	—	Pink.	—	—	—	?
60	6	3	—	—	—	Red.	—	+	?	+
61	6	4	—	—	—	Red.	—	+	—	Bone cells.
62	6	8	+	+	+	Red.	+	+	+	+
63	6	10	+	+	—	Cong.	+	+	+	+
64	6	14	+	+	—	Pink.	+	—	+	—
65	6	19	?	—	+	Pink.	—	+	+	+
66	6	29	Slight.	+	+	Pink.	+	+	+	+
67	6	38	Moderate.	+	—	Pink.	+	+	+	?
68	6	49	Moderate.	+	+	Red.	—	+	+	—

Explanation: + present. — absent. ? doubtful. Cong., congested.

Particular attention is called to the fact that wherever there has been the formation of osteoid tissue in the raised periosteum upon the superficial surface of the cortex, there also has been superficial lacunar absorption of the cortex. In most of the cases there is also absorption of the cortex proper.

In all the specimens the medulla is cellular, in some to a greater extent than in others. Whether this fact is due to the operation or normal to the animal operated upon it is impossible to state, but the fact that, in some of the specimens, the marrow is more cellular toward the cortical edges and yellow in the centre appears to point to some influence upon it of the injury to the bone. Six sections of this series contain large multinuclear cells with abundant protoplasm. They are identical in appearance with osteoclasts, but whether they are osteoclasts or myelocytes it is impossible to prove. Indeed, the two may be identical in fact as well as in appearance. In two specimens in particular (Experiments 60 and 61) there was a condition in the marrow that seems to point to the origin of these multinuclear cells. The medulla of Experiment 60 contains cells of varying sizes: some small round cells with deeply staining nuclei (occasionally eccentrically placed), others with larger vesicular nuclei, and, finally, very large cells with an abundance of protoplasm and four to ten rather large, vesicular nuclei. The medulla of Experiment 61 contains numerous round or oval cells of varying size, with small or moderate-sized, round, well-staining nuclei which, especially toward the ends of the bones, exhibit slight increase in protoplasm and an apparent division of the nuclei into two or four parts.

In four of the experiments there was a formation of osteoid tissue in the medullary cavity, usually moderate in amount, and only in cases where the sawcuts were quite deep. Experiment 68 would appear to be an exception, since the celloidin sections contain a large amount of trabeculæ in the medullary cavity and shallow cuts. In this case the section retained for grinding probably demonstrated the cut to have been quite deep. However, as the specimen was not ground, this is offered merely as an assumption and not as a fact. In a specimen from an experiment of thirty-eight days' duration the defect in the bone is filled in by osteoid trabeculæ, the spaces between which communicate with the spaces caused by absorption of the old cortex.

SERIES 7.

In this series an effort was made to determine whether an animal substance (catgut) placed under the periosteum would excite the formation of bone when not associated with injury to the cortex. The periosteum was raised by an incision along the side of the bone and a coiled strand of No. 2 catgut slipped under it. The wound was then sutured. There were but two operations in this series, and there is a microscopic description of only one of these. In both the macroscopic and microscopic sections there was no new bone formation under the raised periosteum. Microscopically, the catgut is seen to be encapsulated by fibrous tissue and is undergoing partial absorption by leukocytes. Under the portion of the periosteum that still remains attached there is a thin deposit of quite dense new bone which is undergoing absorption on its superficial surface. There is no new bone formation in the medullary cavity, but there is some superficial and slight interstitial absorption of the cortex.

SERIES 8.

This consisted of one successful operation, fifty-one days in duration, in which the periosteum was raised and celloidin, hardened in bichloride alcohol, placed under it to determine if an unabsorbable material would excite bone formation. The celloidin must have slipped from underneath and worked its way above the periosteum, as seen by the microscopic sections. The periosteum shows cellular proliferation, but there is no osteoid bone formation. There is some superficial but little interstitial absorption of the cortex. The marrow is red, but there are no osteoid trabeculæ in the medullary cavity.

SERIES 9 AND 10.

Each consisted of one operation in which the periosteum was raised without injury to the cortex and allowed to fall back into place. In one there was no effort made at hemostasis in the hopes of having a clot form under the periosteum. In the other hemostasis was as perfect as possible.

The results are the same in both. There is superficial absorption of the cortex by osteoclasts, slight cellular proliferation in the periosteum, but no signs of osteoid bone formation. There is but slight, if any, interstitial absorption of the cortex. The marrow of injured and uninjured bone is red and shows large multinuclear cells.

SERIES 11, 12, 13 AND 14.

These four series are treated together because of the close relationship they bear to one another and because they all have as their object the determination of the action of different conditions upon regeneration, after subperiosteal resection of the tibia according to Nichols' method.

The basis of the operations was a typical subperiosteal resection of the tibia performed in the usual manner, by raising the periosteum and removing a section of bone about one inch in length; then of bevelling off the ends of the fragments to obliterate dead spaces, and, finally, suturing the two surfaces of the periosteum together throughout the entire length of the defect. The different series each consisted of two operations upon dogs and were varied in the following manner:

In Series 11 a thin strip of tibia was left connecting the two ends of the fragments and making a bridge between them. The periosteum was sewed over that.

Series 12 consisted of a typical subperiosteal resection.

In Series 13 the medulla was removed from the bone and placed in salt solution, and, after the remainder of the cortex had been removed, was sutured between the two surfaces of the periosteum.

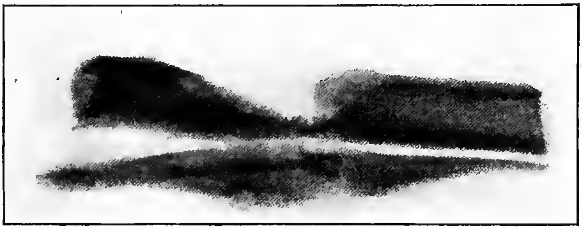

Fig. 28.—Experiment 74, Series 11, fracture forty-seven days Radiogram. Partial superiosteal resection. There is a thin deposit of new bone along the bridge of old cortex, connecting the two fragments of tibia and some over the ends of the fragments. The fibula had been fractured and united with abundant callus.

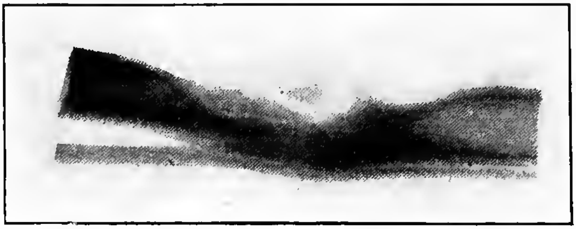

Fig. 29.—Experiment 75, Series 11, fracture forty-four days. Radiogram. Partial subperiosteal resection. There is a deposit of new bone upon the bridge of old cortex connecting the two fragments of tibia, also an isolated island of new bone between the fragments.

In Series 14 thin strips of dried bone were sutured between the layers of the periosteum.

The fractures of the first three series (excepting Experiment 78) were between forty-four and fifty-two days in duration, so that their results can be readily compared. The two of the last series were eight and twenty-nine days in duration.

Macroscopically, in all but the two last operations there was a moderate amount of firmness to the legs in spite of the fact that in several the fibula had been fractured, but reunited to a great extent.

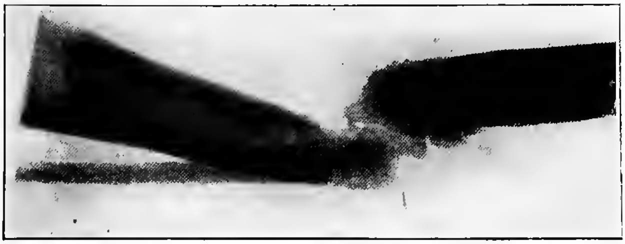

Fig. 30.—Experiment 74, Series 12, fracture forty-six days. Radiogram. Typical subperiosteal resection. There is dislocation of one of the fragments of the tibia. There is no new bone filling in the defect. The fibula is fractured and there is abundant callus upon the fragments.

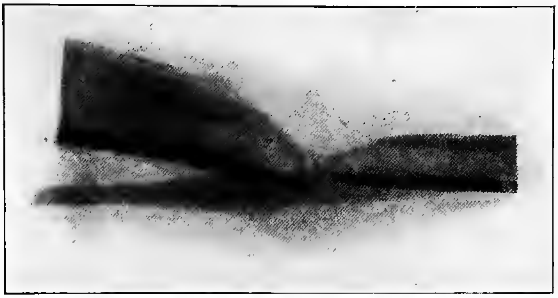

Fig. 31.—Experiment 77, Series 12, fracture forty-four days. Radiogram. Typical subperiosteal resection. There is no new bone filling in the defect of the tibia.

With reference to the amount of osteoid tissue thrown out, as studied by the radiograph, we find in Series 11 (Figs. 28 and 29) a very moderate deposit of new bone over the bridge of tissue connecting the two ends of the fragments and over the ends of the fragments across the medullary canal. With the exception of a small isolated island of osteoid tissue in one, the defect in the bone appears to be devoid of any calcium salts in the form of trabeculæ and appears perfectly clear in the negative.

Series 12 (Figs. 30 and 31) and Series 14 (Figs. 32 and 33) correspond in their results, in that in neither is there any new bone formation excepting at the ends of the fragments of the tibia and across the medullary canals.

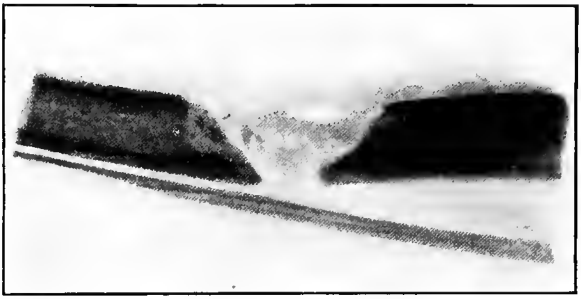

Fig. 32.—Experiment 80, Series 14, fracture eight days. Radiogram. Typical subperiosteal resection of tibia with reposition of bone salts. There is some evidence of the thin section of bone which was sutured between the layers of the periosteum, but no new bone formation in the defect of the tibia.

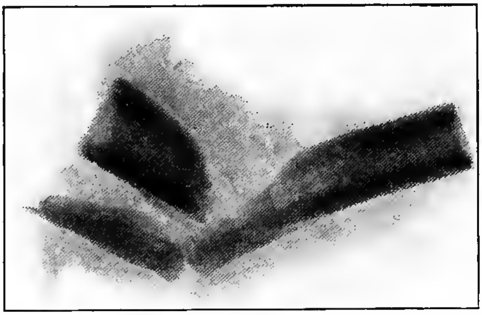

Fig. 33.—Experiment 81, Series 14, fracture twenty-nine days Radiogram. Typical subperiosteal resection of tibia with reposition of bone salts. There is no new bone formation filling in the defect of the tibia. There is a fracture of the fibula with angular displacement

The two specimens from Series 13, in which the medulla was replaced between the layers of periosteum, exhibit a remarkable exception to the above results. The radiograph from Experiment 78, which was only thirty-one days in duration (Fig. 34), although taken at an incorrect angle so that the tibia overlaps the fibula, nevertheless demonstrates

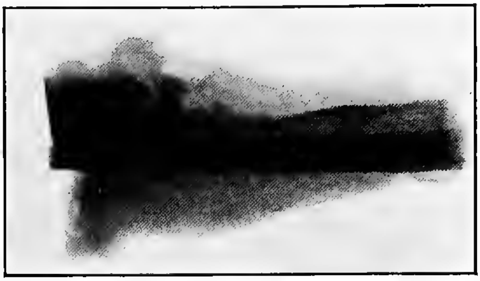

Fig. 34.—Experiment 78, Series 13, fracture thirty-one days. Radiogram. Typical subperiosteal resection of tibia with reposition of medulla. The tibia partially overlaps the fibula, which is intact. There is a fair-sized growth of new bone connecting the two fragments of the tibia, and partially filling the spaces from which the section of tibia had been removed.

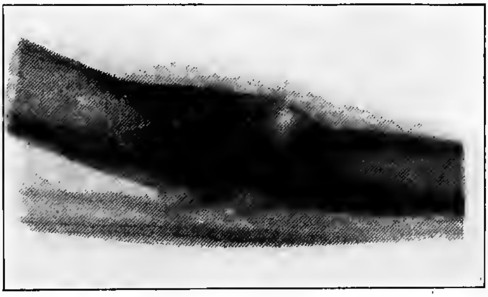

Fig. 35.—Experiment 79, Series 13, fracture fifty-two days. Radiogram. Typical subperiosteal resection of tibia with reposition of medulla. The defect in the tibia is filled with an abundant growth of osteoid tissue, which is partially divided by a strip of cartilage (light streak). The fibula is intact.

clearly a bridge of osteoid tissue extending from one fragment to the other. Its depth cannot be properly estimated from the picture, but microscopic and longitudinal sections show it to be considerable. The most remarkable radiograph is from Experiment 79, which was a fracture of fifty-two days' duration (Fig. 35). Here we see the defect in the tibia more than completely filled by osteoid tissue, so that it is thicker than the bone itself. It is broken by a couple of light streaks which, on cross-section, are found to be cartilage. The animal had perfect use of the leg, which in all respects was absolutely firm.

Longitudinal sections, in all cases, confirm the *x*-ray findings with reference to the new bone formation, but showed that in most of the cases the light areas between the ends of the fragments consisted of cartilage or dense connective tissue, which in some instances was found to be fibrocartilage.

The microscopic sections serve to accentuate some portions of the process that are not brought out by the gross examinations. Foremost is the fact that in almost every instance there is a thin lid or cap of osteoid tissue covering the exposed ends of the medullary cavity of the fragments of the tibia. The process of osteoid bone formation does not appear to extend far up into the cavity. The marrow is more cellular than usual, especially near the ends, but not as cellular as in the experiments of previous series. The ends of the bone almost invariably show lacunar absorption by osteoclasts. There is usually, also, a moderate osteoid deposit on the periosteal side of the fragments.

Cartilage is the dominant tissue filling the defect in the tibia. It is either hyaline cartilage or fibrocartilage. The hyaline cartilage is usually found nearer the ends of the fragments or in relation with forming osteoid trabeculæ. Cartilage is even found in Experiment 79, but here it is in the form of narrow strips surrounding large masses of spongy osteoid tissue. They are evidently the remnants that are undergoing conversion into osteoid tissue.

In these experiments the conversion of cartilage into osteoid tissue more closely resembles embryonal bone formation or that seen at the epiphyseal ends of a growing bone than that in any of the previous series. The cartilage near the ends of the fragments, usually hyaline in character and often taking a very blue stain, most frequently undergoes a rather marked change, the cells becoming arranged in rows or columns with narrow strips of clear matrix between them. Then there is absorption of this matrix and a penetration by bloodvessels. At the same time there is calcification of the remaining narrow strips of cartilage. This is followed by a deposition of more osteoid tissue in lamellæ. The latter tissue takes a deep-red stain and very closely resembles true osseous tissue. This is the typical appearance of almost all the trabeculæ, viz., a central portion containing cells irregularly disposed and taking a bluish stain, and an outer portion consisting of several lamellæ of dense tissue with an orderly arrangement of the cells, the whole taking a deep-red eosin stain. In the experiments of Series 13 the spaces between the trabeculæ filling in the defect in the bone are exceptionally vascular, containing many thin-walled bloodvessels and much free corpuscular blood. The marrow contained in them is very cellular. This is the fact that appears as if it may have direct bearing upon the influence exerted by the medulla placed between the layers of the periosteum. This cellular marrow contains abundant osteoclasts which have, throughout the experiments, appeared to be in close connection with the early deposition of osteoid trabeculæ. They are evidently medullary in origin and may be abundant in these spaces because they may have developed in their present situations.

The experiment of eight days of Series 14 was very interesting from the microscopic standpoint. The centre of the defect is filled with blood clot, but toward the ends of the fragments of the tibia it is replaced by cellular connective tissue, which appears to take origin from the medulla. It contains many round or oval cells that resemble closely the normal marrow cells. It is quite vascular, the vessels also appearing to come from the marrow, which is very vascular. The endosteum and periosteum have already given rise to osteoid trabeculæ on the internal and external aspect of the cortex.

The section of bone placed between the layers of the periosteum shows slight absorption in places, but no deposition of new bone on its surface. This may be of some significance in showing the necessity of the presence of living bone cells in order to have a deposition of bone upon loose fragments. In all the specimens of all the series the cortex exhibits very marked absorption.

The fibula has also shared in the activities of the bone even in cases where it was not fractured. The cortex invariably exhibits absorption to a marked degree, and there is frequently a deposition of osteoid trabeculæ even in the absence of fracture. Where there is not an actual deposit of bone, there is marked superficial lacunar absorption of the cortex and proliferation of the deeper layers of the periosteum.

The striking feature of the results of these experiments is the remarkable growth of osteoid tissue filling the defect of the tibia of those cases in which the medulla was replaced between the layers of the periosteum.

COLLECTIVE REVIEW OF SAWCUT EXPERIMENTS.

Reviewing collectively (Chart 7) all the sawcut experiments, that is to say, the experiments in which the conditions governing repair could be definitely controlled, we find that the first sign of activity on the part of the bone occurs in the form of absorption of the cortex. This is found on the third day in Experiment 51, Series 5, and Experiment 60, Series 6. The probable reasons why this is the earliest activity noticed are that none of the experiments in which the sawcut entered the medulla were shorter in duration than five days, and that in both the experiments mentioned

CHART 7.

Number.	Series.	Days.	Trabeculæ in			Absorption of cortex.			Osteoblasts in cortex.	Marrow.	Direction of medullary spaces between sawcut trabeculæ with reference to the axis of the bone.	Communications between old Haversian canals of cortex and those between sawcut trabeculæ.
			Periosteum.	Sawcut.	Medullary cavity.	Sawcut.	Interstitial.	Surface.				
59	6	2	—	—	—	—	—	—	—	Pink.	A	A
51	5	3	A	—	—	—	+	—	+	Sl. cong.	A	A
60	6	3	—	—	—	—	+	?	+	Red.	A	A
61	6	4	—	—	—	—	+	—	Bone cells.	Red.	A	A
28	3	5	—	—	+	—	—	—	+	Pink.	A	A
42	4	5	A	+	+	—	+	—	—	Red.	L	—
52	5	5	A	—	—	—	+	—	+	Sl. cong.	A	A
29	3	7	—	—	+	—	—	—	+	Pink.	A	A
43	4	7	A	+	+	?	+	—	—	Red.	L	—
30	3	8	—	—	+	—	—	+	—	Red.	A	A
62	6	8	+	Beg.	+	+	+	+	+	Red.	Indifferent.	
31	3	9	—	½	+	—	Sl.	+	—	Red.	L	—
44	4	9	A	+	+	—	—	—	?	Red.	. . . . . .	?
32	3	10	+	Few.	+	+	+	+	—	Red.	L	—
63	6	10	+	Beg.	—	+	+	+	+	Congested	Indifferent.	
33	3	11	+	+	+	—	+	—	—	Red.	L	—
34	3	12	+	Few.	+	+	+	+	—	Red.	L	—
45	4	12	A few.	+	+	+	+	+	+	Red.	L	+
53	5	12	A +	+	+	—	+	—	—	Pink.	Indifferent.	+
64	6	14	+	—	—	+	—	+	—	Pink.	A	A
35	3	15	+	+	+	—	+	+	—	Red.	L	—
46	4	15	A	+	+	+	+	+	—	Pink.	L	Beginning.
36	3	16	Sl.	+	+	+	+	+	+	Red.	L	—
37	3	17	+	+	+	+	+	+	+	Red.	L	—
38	3	19	+	+	+	—	+	—	—	A	L	—
47	4	19	A +	+	+	—	+	+	—	Red.	L	—
65	6	19	—	—	+	—	+	+	+	Pink.	A	A
39	3	22	+	+	+	+	+	Sl.	—	Red.	L	+
48	4	23	A	+	+	+	+	+	+	Red.	L	+
54	5	24	A	—	+	—	+	—	+	Red.	A	A
49	4	28	A +	+	+ +	—	+	+	+	Red.	L	+
66	6	29	+	+	+	+	+	+	+	Pink.	Indifferent.	+
55	5	30	A	?	—	+	+	—	?	Red.	?	?
Ground.												
40	3	32	+	+	+	—	+	—	—	Red.	L	+
56	5	35	A	—	+	+	+	+ d. f. c.	+	Red.	A	A
67	5	38	+	+	—	+	+	+	?	Pink.	=	+
57	5	45	A	+	+	—	+	?	?	Red.	=	+
Ground.												
50	4	49	A	+	+	—	+	—	+	Pink.	=	+
68	6	49	+	+	+	—	+	+	—	Red.	=	+
41	3	70	+	+	+	+	+	—	—	Red.	=	+
58	5	70	A	+	+	+	+	+	—	Red	=	+

Explanation: + present. — absent. A, primary tissue absent. ? doubtful. Beg., beginning. Sl. cong., slightly congested. ½, half its depth. Sl., slight. d. f. c., distance from cut. L, at right angles. = parallel.

the periosteum had been removed from the bone, although in one case it was allowed to fall back into place.

When we come to the fifth day we are struck with the fact that in those experiments in which the medullary cavity had been entered there is present in it a new formation of osteoid tissue (Experiments 28 and 42). This appears to be greater in amount in the experiments in which the periosteum has been removed from the bone than in those in which it is left remaining. In the former (Series 4) the medullary trabeculæ enter the sawcut as early as the fifth day, whereas in the latter this does not occur until the ninth day

Absorption of the sides of the sawcut does not appear until the eighth day (Experiment 62) and does not seem to bear any definite relation to the form of experiment performed. It is not constant in its occurrence.

The most important factor that can be noted when considering these experiments is that, with but two exceptions in Series 5, where the periosteum was removed and only shallow sawcuts made in the bone, and two exceptions in Series 6, where the periosteum was raised from the bone and shallow cuts made, the sawcut in every experiment after the eighth day contains osteoid trabeculæ. In *every* operation in which the medullary cavity has been entered the trabeculæ in the sawcut take origin in the medullary canal. In every experiment up to the thirty-second day, in which the medullary cavity has been entered and there are trabeculæ in the sawcut, the direction of these trabeculæ is at right angles to the axis of the bone. In those experiments in which the medullary cavity has not been entered there is usually no definite direction to the trabeculæ, the spaces between them being round.

In every experiment after the thirty-eighth day, in which there are trabeculæ in the sawcut, the direction of the spaces and trabeculæ is in the line of the bone, and there are communications between the spaces which separate the trabeculæ and the Haversian canals of the cortex

DEDUCTIONS AND CONCLUSIONS

Having given a resume of the experiments, the details of which are to be found at the end of the paper, we may now proceed to formulate our deductions and conclusions When we consider the first two series in which complete fractures were studied we have the condition which exactly simulates that encountered by the clinician. As mentioned previously, this is not the ideal state in which to study the activities of the individual portions of the bone, but since it is the form in which injury to the bone most frequently is met, we should have some definite ideas as to the general process.

Dupuytren has classified the process of the repair of bone into five distinct stages, based upon macroscopic findings. The periods he distinguishes are, in brief, as follows:

1. From the first to the tenth day: This is the period immediately following the fracture and is associated with the infiltration and engorgement of the tissues by blood. The resulting coagulum is absorbed between the ends of the fragments and there is a viscid, gelatinous material poured between the ends which (he says) plays an important part in the final repair of bone. The medulla becomes swollen and hardened and of a reddish, fleshy, or pulpy appearance as a result of a sort of gelatinous infiltration.
2. From the tenth to the twentieth or twenty-fifth day: In this period the callus, which is mostly cartilaginous, takes definite shape, the soft tissues becoming isolated from it. The medullary membrane obliterates the canal and proceeds to cartilage and osseous tissue formation. The whole mass constitutes the provisional callus.
3. From the twentieth to the thirtieth, fortieth, or sixtieth day, according to the rapidity of the work of reproduction, the age, constitution, and health of the patient. In this period the cartilage becomes converted into bone, the entire

provisional callus becoming spongy bone. The muscles and tendons become free, but there is absolutely no union between the ends of the fragments.

4. From the fiftieth or sixtieth day to the fifth or sixth month: In this period the provisional callus becomes condensed from spongy to compact bone and the medullary canal is obliterated by osseous matter of greater or less density. The substance between the fragments is reduced to a mere line of a different color from the bone itself; it gradually assumes more consistency, loses its color, and ultimately, toward the end of the period, becomes ossified; the definite or permanent callus is then formed.

5. From the fourth or sixth to the eighth or twelfth month: During this final period the temporary callus is absorbed, the medullary cavity becomes reëstablished, the muscles and tendons perfectly free and the union is complete.

This division of the subject is very convenient and very logical when we recall that it is based upon macroscopic findings, but in the light of the present experiments it appears as if a slightly more accurate division of the process can be based upon both macroscopic and microscopic examinations. According to this method we venture to divide the process into the following periods:

1. From the time of fracture until about the fourth day may be considered the period of *infiltration.* The bone has been fractured, this being associated with free hemorrhage from the bone, medulla and all the injured soft tissues in the neighborhood. (The periosteum may also assist in the effusion of blood, but I have never found it to bleed in any operation upon a normal animal where it has been cut with a knife.) The cortex of the bone is usually fragmented, the fragments lying loose across the line of fracture, and some, at times, being driven into the medullary tissue. The blood which infiltrates all the tissues and is found at the site of fracture loses its corpuscular nature within forty-eight hours, and, in its stead, we find fibrin first as a loose

network and then in dense masses. It is probably this which is described by Dupuytren as the gelatinous material appearing early between the ends of the fragments. All the tissues are moderately infiltrated by polymorphonuclear leukocytes. This infiltration disappears by the fourth day. Toward the end of this period the fragments do not appear to be quite as movable as at the time of fracture, this being due either to the edematous and engorged condition of the surrounding tissues or to the masses of fibrin between the ends of the fragments gluing them together. After the second day there is beginning cellular proliferation of the medulla and of the deeper layers of the periosteum. There is connective hyperplasia of the medulla.

2. From the fourth to the ninth or twelfth day may be considered the period of *temporary callus formation.* During this period we see a gradual absorption of the products of hemorrhage in the soft tissues, which is replaced by a connective-tissue hyperplasia. The periosteum, which was beginning to show proliferation, now becomes very thick. Its deeper portions consist of large polygonal cells which, at some distance from the site of fracture, secrete a homogeneous matrix in lines at right angles to the cortex, which matrix becomes calcified to form osteoid trabeculæ. Some of the cells are included in this mass and become bone cells. The remainder arrange themselves as a layer upon the trabeculæ as the osteoblasts. The spaces between the trabeculæ become vascularized and medullary spaces are formed.

Nearer the site of the fracture the cells of the periosteum in many cases appear to be embedded in a homogeneous matrix, which is more hyaline in character and does not appear in streaks, but is diffuse. It shows no tendency to calcification, and is probably the earliest stage of cartilage formation.

In the medullary cavity the endosteum undergoes the same proliferation that the periosteum has displayed, with a similar formation of osteoid trabeculæ at some distance from the site of fracture. The medulla becomes hyperplastic, with a decrease of the fat cells and an increase in the connective tissue and medullary cells. Between the ends of the fragments there is usually fibrin with gradually increasing connective tissue and, in some cases, cartilage.

All the processes described increase, so that at the end of this period there is a fair amount of callus present, often, when the position is fairly good, forming a complete sheath of the fragments. The callus usually consists of osteoid tissue on both fragments, with cartilage at the line of fracture; osteoid tissue in the medullary canal, and connective-tissue or cartilage between the ends of the fragments.

3. From about the twelfth to about the eighty-fifth day may be designated the stage of *reorganization*. During this period there is augmentation of the callus where most needed and absorption in the places where callus is not required. Osteoclasts are to be found in close proximity to osteoblasts, the one causing absorption along definite lines, while the other strengthens the weakened points along the new lines. The deeper portions of the periosteal callus become channelled in a direction parallel with the old cortex, while the more superficial portions under the periosteum and along the lines of stress and strain become very dense by the action of the osteoblasts. The cartilage, which has assumed definite shape and size according to the amount of displacement of the fragments (especially angular), becomes gradually converted into osteoid trabeculæ. Ossification originally occurred throughout the mass of cartilage, but as the latter becomes more localized the conversion occurs only at the edge of the mass. The trabeculæ which are in the medullary cavity also undergo some absorption. Across the line of fracture there is no osteoid tissue, but merely dense connective tissue or, at times, cartilage. During this period the cortex suffers great absorption by osteoclasts, the widened spaces, in many cases, again being lined with osteoblasts.

These spaces communicate freely with the spaces between the medullary and periosteal trabeculæ.

4. From the eighty-fifth day to the end of the longest period of any of these experiments is the stage of *permanent callus formation*. During this period there is extensive absorption of all the callus and cortex, with a reapposition of apparently much denser bone in the dilated spaces. We now notice the formation of distinct lamellæ in the trabeculæ. What is most important and the part that distinguishes this period, is the fact that osteoid tissue appears across the line of fracture. It is at first very porous and its spaces communicate with those of the cortex. This latter becomes denser until it appears even denser than the original bone. The cartilage gradually disappears, the time at which this occurs varying according to the amount present.

5. As far as I am concerned this is only theoretical, but it seems that there must be a period, as described by Dupuytren, in which, in simple fractures, there is complete absorption of all the provisional callus and a complete restoration of the medullary canal. The latter of these experiments show that there is a tendency for this to occur. This should be known as the period of *absorption*.

It will be seen from the above that the views herein expressed and those formulated by Dupuytren coincide in many respects. His idea of a provisional and a permanent callus is most heartily endorsed. In none of the experiments and in none of the cases that I have seen clinically has there ever been, as claimed by Hamilton, Gaillard, and Paget, what would appear to be direct union between the ends of the fragments without the interposition of callus. The fact that in fractures that had apparently united perfectly there is, up to about three months, nothing but cartilage and connective tissue across the line of fracture, is one that is never considered, although it may be of considerable clinical importance. Jacquemin evidently recognized the clinical importance of the fact that the callus was pliable for a considerable length of time, for he succeeded in correcting angular displacement by extension and gentle pressure several months after the apparent union of the fracture. That a condition similar to that found in these experiments occurs also in human beings is beautifully substantiated by a case encountered in one of the dispensaries of this city. The patient was a young man who had sustained a concussion of the brain and a fracture of the ulna below the olecranon. He recovered from the concussion and the fracture united completely in extension. At the end of six weeks he presented himself for the first time at the dispensary for treatment for ankylosis of the elbow-joint. There was apparently perfect union of the fragments in good position accompanied by ankylosis of the elbow-joint in extension, there being only a very limited range of motion. *X*-rays (Fig. 36) show clearly the line of fracture with the fragments in perfect

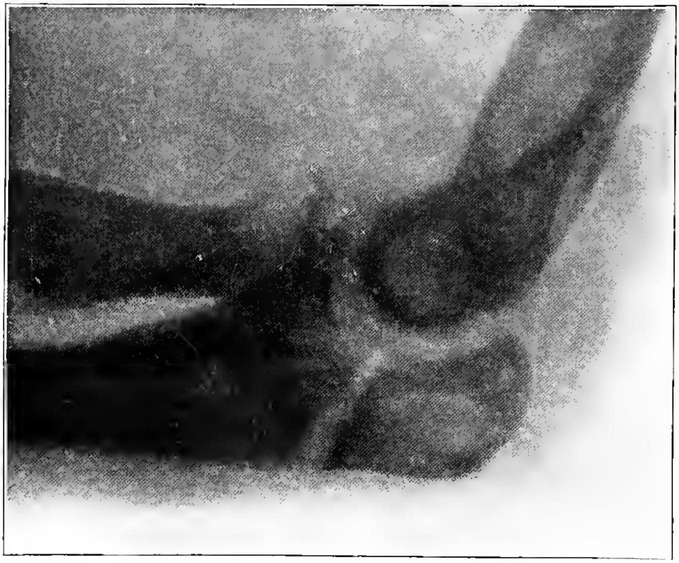

FIG. 36.—Radiogram of a fracture of a human ulna of six weeks' duration showing the line of fracture still plainly visible, although the fracture was clinically perfectly united.

position. Had the bone united by osseous tissue this line would no longer have been visible. The man was etherized and the adhesions in the elbow broken up and the arm placed in acute flexion without any injury to the fracture whatever.

The proper explanation for the appearance of cartilage in the callus appears to me extremely hard to arrive at, if at all possible. Should we consider it a reversion to the embryonal state we must naturally ask why it occurs only in the region of fracture and not throughout the periosteal and medullary callus, and why it does not appear in a single one of the sawcut experiments. If it is due to motion of the fragments alone, how can we explain its presence in the medullary cavity of a greenstick fracture? What appears to be the most rational explanation of its appearance is that it is due to a number of factors, each with its influence, and each, at times, acting independently of the others. Probably the first and foremost factor is defective nutrition and an insufficient blood or lymph supply to carry lime salts, so that the process can proceed at once to osteoid tissue formation. Angularity of the fragments with improper fixation is the most important clinical factor, causing an increase in the inflammatory conditions surrounding the parts with consequent fibrosis, and thereby impairing nutrition. The idea expressed by Matsuoka, that it is due to the amount of pressure exerted by the bandage, appears untenable. A final theory which has suggested itself as a mere possibility is that the formation of cartilage bears some relation to the fibrin between the ends of the fragments. In several specimens the cartilage was always in close proximity to the fibrin, which several times almost appeared to show stages of transition into cartilage. It may be that cells derived from the periosteum penetrate the masses of cartilage and secrete a substance which causes a chemical change to occur in the fibrin, with the resulting formation of cartilage. While I doubt if this is the case, the appearances before mentioned compel the suggestion.

With reference to the influence of the soft tissues in the repair of fractures, I believe with Macewen, that it does not assist but may hinder union. The only possible assistance to union appears to me to be the infiltration and induration that occurs in the tissues which may act as a tempoary splint until the formation of the temporary callus. I need not mention that most of the above findings are at variance with the older views of Bordenave, Hunter, Haller, Dethleef, and others.

Leaving the subject of complete fractures as found clinically and considering it when the conditions are ideal as to fixation, etc., we see that the process is, with comparatively few exceptions, practically identical. The subject can be divided into the same periods as in the case of the clinical fractures, but the time of the periods changes somewhat owing to changed conditions. In the first place, when the sawcut operations were performed, all hemorrhage was checked before the wound was closed and the periosteum was but little damaged, not being torn and shredded as in complete fractures ordinarily encountered. This has a marked influence upon the first and second periods. During the first period the large masses of fibrin are not to be found, but the blood retains its corpuscular form for a longer period. There is a rapid reaction upon the part of the medulla which throws out connective tissue, rapidly filling the sawcut. This tissue being mostly medullary in origin, is a fertile soil for the ingrowth of osteoid trabeculæ. In addition, it is subject to no pressure and mobility to cause it to become condensed and avascular.

The importance of all these facts is readily seen when we come to the second stage of the repair. This appears in the medulla at the usual time, but in the periosteum it is late in its development, not appearing until the tenth day. The second and third periods differ very essentially from the clinical fractures in that, instead of dense connective tissue, osteoid tissue is found in abundance between the ends of the cortex. It differs also to some extent in the late appearance of reorganization, which is mostly localized to the old cortex and the trabeculæ in the sawcut. This is readily explained by the fact that there is no need for reorganization elsewhere, there being no point of greatest stress, etc. The influence upon the callus exerted by stress and strain has been very thoroughly worked out by Wolff, and I believe these experiments go far toward proving their importance and accuracy. When, in these experiments, absorption of the medullary and periosteal trabeculæ does occur, its object appears to be mostly the reduction of the bulk of the callus, which has become excessive owing to the abundant callus in the sawcut. In the simple complete fractures probably the entire stage of reorganization is for the sole purpose of making the new bone mechanically perfect. This absorption in the new lines of the bone is shown in the deeper layers of the periosteal callus and in the cortex, etc., of several specimens (Series 2) with angular displacement, where there is rarefaction of the cortex at the site which is ultimately to carry little strain (owing to the new callus occupying its former position), and condensation of the medullary trabeculæ where the new line of the bone passes through it. Thus we see structures that are normally dense becoming absorbed and structures that are normally absorbed becoming permanent.

There is probably no better place than this to call attention to a fact concerning the formation of periosteal callus that I have noticed in almost all the experiments. Almost without exception preceding or simultaneous with the formation of periosteal osteoid trabeculæ there is superficial absorption of the cortex by osteoclasts. Hofmokl has attributed the formation of pits in other portions of the bone to proliferated bone cells having several nuclei, but I have been able to find no evidence of this occurring and believe that they are caused by osteoclasts. The origin of these osteoclasts I am unable to explain. They may be medullary in origin and have the faculty of wandering through the tissues or of being carried through the blood, or they may possibly originate in the periosteum itself. To determine whether they are carried through the blood, I had differential blood counts made of a number of patients suffering from fractures, and in none were multinuclear cells found, the only change being a lymphocytosis. The fact nevertheless remains that there is superficial absorption of the cortex preceding the deposit of trabeculæ. It may be that the function of these cells is to act upon the calcareous salts, rendering them in a soluble state, to be redeposited by the action of the osteoblasts in the vicinity. Their presence cannot be attributed to necrosis of the bone, for they were found under the portions of the periosteum, which had not been disturbed, and the bone was perfectly healthy in appearance.

The fourth stage of repair is very beautifully illustrated in these experiments. The process of the union of the ends of the cortex with the trabeculæ between them and the change in direction of the canals between the trabeculæ has already been sufficiently brought out so as not to require repetition (Fig. 19). These experiments have given a new importance to the medullary trabeculæ and medullary tissue. We have seen that all the trabeculæ in the sawcut have been medullary in origin, which fact was also noticed in the complete fractures of the clinical variety. We have seen that the medullary tissue itself assists in the formation of trabeculæ by forming a framework upon which they can develop, if, indeed, the medullary tissue itself is not capable of bone formation. These experiments demonstrate that cartilage formation must be due to some extrinsic influence, since it is not present in any of the sawcut experiments. They also demonstrate that hard and fast rules for the repair of bone cannot be laid down, owing to the varied conditions under which union takes place.

When we come to consider the other sawcut experiments in which an effort was made to isolate different portions of the bone we see that the endosteum is the most active individual portion of the bone, then the periosteum, and finally the cortex; but that every portion is capable of assisting the other portions and, if necessary, performing the whole work alone. Many years ago Hilty proved the activity of the endosteum by his experiments, by which he obtained a growth of osteoid tissue in the medullary cavity by maintaining a constant pressure on the periosteal side of the cortex and also by several other experiments somewhat similar to those performed by the writer.

The osteogenetic property of all portions of the bone has a very important clinical bearing, for it means that in operations upon bone, nothing whatever should be removed excepting what is absolutely diseased, for even loose fragments of bone, as shown clinically by Macewen and experimentally in this work, can assist in the formation of callus. The medulla has too long been held in more or less contempt and has been carelessly destroyed on many occasions when it could have been saved.

Finally, in considering the possibility of regeneration of bone after subperiosteal resection the experiments cited herein would seem to prove the claim of Ollier that complete regeneration of the bone is possible after subperiosteal resection and would tend to place the operation of Nichols upon a still firmer basis. The precaution taken by Nichols to wait until periosteal trabeculæ can be demonstrated before removing the shaft is based upon sound principle, for I have never found osteoid trabeculæ in the periosteum that has been raised from the bone and is not in contact with osseous material. Should the periosteum be removed before it has begun the formation of osteoid trabeculæ the probable result would be that found in these experiments, viz., the formation of cartilage. This would doubtless later ossify, but the process would be very slow. These experiments appear to offer two choices in cases where it would be possible to do the operation very early. In the first place a bridge of healthy bone can be left between the two ends as tried by Nichols and proved of value by these experiments, or, what appears to be still more efficacious, healthy medulla, if obtainable, can be placed between the two surfaces of the periosteum. I have not had time to experiment with the transplantation of medulla of different species between the layers of the periosteum, but if this should prove feasible a great advance in bone surgery would be achieved. The exact modus operandi of the medulla in these cases is difficult to explain excepting that it may mean the introduction of tissue that has potent osteogenetic properties, is easily vascularized, and contains many osteoclasts to obtain calcium salts from the neighboring bone. Whatever may be the cause it certainly appears to assist in the regeneration and deserves further trial and study. The importance of the presence of calcium salts suggests the placing of these in powdered form, between the layers of the periosteum.

As a final word the following conception of the bone may be ventured in an effort to render more comprehensible the claims made above. If we consider the bone in the light of an organ similar to other organs, for instance, lymph glands, we can describe it as having a cortical and medullary portion and being surrounded by a capsule. The capsule corresponds to the fibrous layer of the periosteum. The cortex corresponds to the deeper layer of the periosteum, the endosteum, the cellular tissue in the Haversian canals, and the bone cells. The medulla consists of loose connective tissue and cells of various size and shape, exhibiting blood-forming properties. In portions it is extremely infiltrated with fat. Some of its cells are multinuclear and appear to have phagocytic properties.

With the above as a working hypothesis we can explain upon physical grounds the varied activities of the different portions of the cortex. The bone cells being within very close quarters, and separated from most stimuli by dense calcareous walls, are slow to react and can only form bone when liberated from their close confines. The periosteum has much more freedom to expand and is much more exposed to external stimuli, so that it responds more quickly. It has not, however, owing to the fibrous capsule, the freedom from pressure that the endosteum enjoys, and therefore is not quite as active as that membrane. In addition, the close proximity of the endosteum to the vascular portion of the organ gives it a richer nutrition and we have more rapid growth.

Upon the basis of this theory we may explain the presence of osteoclasts upon the superficial surface of the cortex as being periosteal in origin, since all the soft tissues of the bone are related, one to the other, and of common origin. Therefore, as the medulla is capable of forming mononuclear and multinuclear cells of different nature so also may the periosteum be capable of forming osteoblasts and osteoclasts.

SUMMARY OF CONCLUSIONS.

1. In regeneration of bone all the various elements, viz., periosteum, cortex, endosteum, and marrow, assist in the process.
2. In their activities they appear to bear more or less reciprocal relations to one another, so that the absence of one can be compensated for by the others.
3. The activities of the endosteum and the medulla can be brought into play by slight stimulus.
4. The periosteum, even when proliferated, is incapable of forming osteoid trabeculæ unless in close contact and union with previous bone formation or calcareous salts.
5. Periosteal bone formation is usually preceded by superficial absorption of the cortex through the action of osteoclasts. These possibly liberate lime salts in the tissues, for the osteoblasts to again precipitate in the surrounding hyaline matrix.
6. Cortical bone-forming activity does not manifest itself

until after considerable absorption of the cortex has occurred, so that it may be due to the liberation of bone cells which take on new activity and again precipitate the liberated calcium salts in new situations; or it may be due to the opportunity for the ingrowth of endosteum into the widened spaces.

7. In unimpaired regeneration of defects of bone the defect is filled with trabeculæ and spaces that originally run at right angles to the line of the bone, but rearrangement occurs in the later stages, with a change in the directions of the canals of the new bone. This is the normal function of cortical activity.

8. The medulla assists in the formation of osteoid trabeculæ by means of a skeleton framework along which the trabeculæ form and also, possibly, by the activities of some of the marrow cells.

9. The deposit of calcium salts in certain lines to form trabeculæ is not due to bloodvessels, as the spaces between the trabeculæ, until some time after they have been formed, do not contain bloodvessels.

10. Cartilage is a pathological but constant feature of the repair of complete fractures. It is not found in normal regeneration free from extrinsic influences.

11. The formation of cartilage may have some direct or indirect relation to the masses of fibrin found in the region of the line of fracture or may represent a retarded development of osteoid tissue due to poor nutrition.

12. In complete fractures the amount of cartilage present depends mostly on the angularity of the fragments, and it is always found in a wedge shape on the internal side of the angle.

13. Complete fractures are very slow in their final union, so that in the case of vicious union they may be safely rebroken up to nine months and even longer without danger of fracturing the bone in another situation. They may even be made to yield to constant traction in the proper direction.

14. There is usually sufficient callus formed on the internal side of an angularly deformed fracture to almost reëstablish the original line of the bone from one joint to the other, but with commensurate shortening.

15. In subperiosteal fractures the repair is probably mostly from the endosteum and medulla because of the extravasation of blood into the medullary canal, injuring the medulla to a greater extent than the periosteum.

16. In repair of greenstick fractures the endosteum and medulla are probably the dominant factors.

17. Regeneration of the bone after subperiosteal resection is usually cartilaginous in nature when sufficient time has not elapsed before the operation for the formation of periosteal osteoid trabeculæ.

18. A strip of healthy bone, when left between the two fragments of the resected bone, hastens osteoid regeneration.

19. Healthy medulla, when sutured between the apposed surfaces of the periosteum, hastens considerably the regeneration of bone.

20. The process of the repair of complete fractures may be divided into the following five stages:

1. Infiltration.
2. Temporary callus formation.
3. Reorganization.
4. Permanent callus formation.
5. Absorption.

21. Similar stages are probably found in the human subject also, with slight differences, owing to the size and nature of the species.

22. The deeper layer of the periosteum, the endosteum, the tissues lining the Haversian canals and the bone cells are probably all related or identical tissues, exhibiting different activities because existing under different physical conditions.

23. Cartilage is converted into osteoid tissue in several different ways, depending upon the age of the cartilage and the stage of the fracture.

24. Osteoid tissue, when seen in ground section, is not as dense in structure and has a deeper yellow tinge than normal cortex, probably due to some difference in chemical composition.

25. Specimens of bone, with their soft parts attached can be ground to microscopic thinness, but they possess no advantage over decalcified specimens beyond demonstrating canaliculi.

26. Investigations of regeneration of bone, when not accompanied by microscopic study, are untrustworthy and often deceptive.

EXPERIMENTS IN DETAIL.

All the experments were performed under the influence of an anesthetic administered usually by Mr. James Timlin. For the sake of brevity, owing to the length of the experiments, the mode of operating will not be repeated under each operation; therefore one will answer for the particular series, merely the results of the others being given.

SERIES 1.

As stated in the body of the paper, this series was performed upon rabbits and consisted of complete fractures of the foreleg, made by grasping the limb between the fingers and breaking it subcutaneously and then placing it in cotton and a sodium silicate bandage.

Experiment 1. Operation performed May 25, 1905. Animal killed May 26, 1905. Fracture of one day's duration.

Macroscopic Description. The bones were found to have been broken through completely, the end of one of the fragments projecting through the deep fascia. There was a great amount of edema of the soft tissues and some hemorrhage into the muscular tissue. The leg was embedded in the manner described under Technique, and then cut and stained with hematoxylin and eosin, which were the stains used throughout all the experiments.

Microscopic Description. Under low power the sections show an almost transverse fracture, with marked displacement and fragmentation of both bones of the foreleg. The muscular tissue in the immediate neighborhood of the fracture is markedly torn and mingled with fragments of bone. In places, portions of muscles are caught between the ends of the fragments. There is very little free blood present, but between the fragments there appears to be a loose network of fibrin. In the medullary cavity there is practically no reaction. The periosteum is infiltrated in places.

Under high power, the fibrin, muscles, detritus, and all the tissues in the neighborhood of the fracture, are seen to be moderately infiltrated with leukocytes. The specimen illustrates very well the natural union of the two bones laterally by cartilage with the absence of periosteum.

Experiment 2. Operation performed August 7, 1905. Animal killed August 9, 1905. Fracture of two days' duration.

Macroscopic Description. There was no preternatural mobility, so that at least one of the fractures must have been greenstick. On removing the skin there was found slight ecchymosis over the middle of the foreleg at the probable site of the fracture.

Microscopic Description. The fracture was complete at least in one bone, although the bones are in perfect position. The only explanation that seems to offer itself for the perfect position is that one bone may have had only a greenstick fracture. If the apparently complete fracture was greenstick, there must have been only a very slight bridge of bone left, which may have been missed in the selection of specimens for study. That this is probably the explanation is evidenced by the fact that one specimen presents the medulla not broken across, so that there could not have been much displacement.

Under low power the muscles attached to the bone are seen to be infiltrated with blood in the interspaces between the fasciculi, but there is very little injury or fragmentation of the muscles. Much of the blood maintains its corpuscular shape, but in places, between the fasciculi, there is a delicate network of fibrin formed. There is very little inflammatory reaction in the form of leukocytes, but there are some young fibroblasts. Between the fragments and extending along the line of fracture are masses of fibrin intermingled with deeply staining, red, granular masses of debris. There is slight fragmentation of the fractured ends of the bone. In the periosteum, especially in some of the sections, can be noted signs of beginning thickening, there being three or four superimposed layers of long, spindle-shaped cells, with large, oval nuclei.

The hemorrhage in the medullary cavity appears to have run up the side, separating the medulla from the cortex more than tearing up the medulla itself. In some of the sections there are signs of proliferation of the endosteum, especially of the superficial layers, with the formation of fibroblasts or a loose connective tissue. In other sections there is marked proliferation of the deeper layers of the endosteum, with the formation of large, polygonal cells. The marrow is hyperplastic, with a change from the yellow to the red variety.

Experiment 3. Operation performed September 11, 1905. Animal killed September 14, 1905. Fracture of three days' duration.

Macroscopic Description. On removing the skin a very extensive ecchymosis with edema of the entire foreleg was found. The bones were not united, but did not appear to move as freely as when fractured.

Microscopic Description. Sections of the leg show complete fracture of at least one of the bones, with marked fragmentation of the ends of the fragments. There was not much displacement of the ends of the bone. The muscular tissues are infiltrated with young fibroblasts separating the fasciculi, but there is very little free blood present. In the immediate neighborhood of and between the fragments there are masses of fibrin, in some places quite dense, in others forming a looser network, containing in its meshes red blood cells and some necrotic debris.

There is thickening of the periosteum at some distance from the ends of the fragments, mostly of the superficial fibrous layer, which is continuous with the processes between the fasciculi of the muscles. There is also some thickening of the endosteum, especially in some areas, with the formation, in its deeper layer, of polygonal cells of the osteoblastic type with large, oval nuclei. In the medulla also are signs of hyperplasia, with the increased formation of connective tisue and bone-marrow cells, including many eosinophiles. There is a slight decrease in the number of fat cells.

Experiment 4. Operation performed July 27, 1905. Animal died July 31, 1905. Fracture of three and one-half days' duration.

No macroscopic description recorded.

Microscopic Description. There is comminution of the fragments of both bones, with poor position. There appears to be considerable hemorrhage still in the muscular tissue, which is extensively lacerated in the region of the frature. Between the ends of the fragments and extending across the medullary cavity are plugs of fibrin, in places quite dense and in other places in the form of a loose network which contains in its meshes what appear to be red blood cells with a few leukocytes among them.

The periosteum and endosteum appear to be slightly thickened, but the cellular characteristics are destroyed.

Experiment 5. Operation performed May 25, 1905. Animal killed May 29, 1905. Fracture of four days' duration.

Macroscopic Description. There was no union or fixation apparent after removing the dressing. There was some nodulation at the ends of the fragments, which was probably beginning callus. On removing the skin general ecchymosis was found, especially in the dependent portions of the leg and at the joints.

Microscopic Description. There is absence of blood between the muscular fasciculi, its place being taken by an abundance of young connective tissue. Between the ends of the fragments, as if gluing them together, are dense streams of fibrin in which there do not appear to be any free blood or cellular elements (Fig. 1). There are also numerous fragments of bone with some necrotic debris.

The periosteum is markedly thickened, both the superficial fibrous layer and the deep osteoblastic layer, in which are large polygonal cells with large oval or round nuclei. At some distance from the site of fracture, the protoplasm of the cells of the osteoblastic layer appears to become homogeneous and fused with that of the surrounding cells, the whole mass of homogeneous material taking a pink stain, apparently only the nuclei remaining enmeshed in the mass.

On the surface of this material the osteoblasts are arranged in rows. The fusion takes place in narrow lines which appear to unite and form bridges when seen in section (Fig. 2). This tissue is osteoid tissue.

In other areas the protoplasm of the proliferated cells of the periosteum seems to be transformed into hyaline material which does not take the red stain. The cells apparently fuse, but have, as yet, no definite arrangement. They have the appearance of beginning cartilage.

In the endosteum there is very marked proliferation. The deeper layers of polygonal cells are arranged in rows on the surface of the compact bone and, in places, have undergone similar homogeneous transformation and fusion, with the formation of osteoid tissue. The new formation of bone occurs only at some distance from the site of the fracture. The medulla contains many red blood corpuscles and shows connective-tissue hyperplasia, with a decrease in the number of fat cells.

Experiment 6. Operation performed August 23, 1905. Animal killed August 28, 1905. Fracture of five days' duration.

Macroscopic Description. There was no firm union of the ends of the fragments, but there were evidences of callus formation, as the fragments seemed to be somewhat bound down.

Microscopic Description. Under lower powers no free blood but an abundance of connective tissue is seen in the muscular tissue. There is marked displacement of the fragments. The periosteum shows considerable thickening, with the formation of an abundance of osteoid trabeculæ, as far as the ends of the fragments and in the bridge across the line of fracture.

The tissue across the line of fracture is composed mostly of typical cartilage, with its deep-blue staining cell surrounded by a light halo, and then a deep-blue capsule and lighter blue hyaline ground substance. There is a decided tendency toward the formation of twin cells. The cells of the cartilage are very large and very abundant, whereas the ground substance is insignificant.

In areas a transition from cartilage into osteoid tissue can be seen. The cells apparently shrink somewhat, the ground substance becomes more abundant and stains red in spots, while the cells in the intervening areas disappear and their places are taken by an invasion of connective tissue between the trabeculæ. In the younger areas this has not occurred, but large plugs of cartilage can be seen to be surrounded by transitional trabeculæ.

There is considerable proliferation of the endosteum, with the formation of osteoid trabeculæ to the ends of the fragments. It contains no cartilage. The marrow shows connective-tissue hyperplasia. Other elements cannot be distinguished.

Experiment 7. Operation performed August 15, 1905. Animal killed August 21, 1905. Fracture of six days' duration.

Macroscopic Description. There was marked ecchymosis and edema of the lower portion of the leg. There was no firm union, but the ends of the fragments were evidently united by some material that prevented free motion of them.

Microscopic Description. There are osteoid trabeculæ on the fragments, but the most interesting portion of the specimen is the dense mass of fibrin found between the ends of the bone. On its outskirts this appears to be infiltrated by connective-tissue cells, the protoplasm or ground substance of which is of a rather reddish hue. This tissue is, in turn, in juxtaposition with cartilage. On its other side the fibrin is in close relation with a mass of cartilage, which has bands of fibrin coursing through it. The whole appearance raises the possible suggestion that cartilage, which is almost invariably found on the periosteal surface and between the fragments, could be derived from organization of the fibrin by periosteal or connective tissue or osteoblastic cells.

Experiment 8. Operation performed May 19, 1905. Animal killed May 26, 1905. Fracture of seven days' duration. This operation was only a partial fracture with bone forceps.

Macroscopic Description. There was a small nodule of callus about one-fourth inch long at the site of operation.

Microscopic Description. The sections show that a fragment of bone was left in the trough made by the forceps. There is a great abundance of young cellular connective tissue, much of which has the characteristics of granulation tissue, filling in the defect of the bone, in the medullary cavity and outside of the bone. There are dense strands of fibrin at the edges of the bone wound.

On the periosteal surface, at some distance from the bone injury, there are some osteoid trabeculæ, but they are not present in such abundance as in the medullary cavity. The endosteum shows considerable proliferation. Opposite to, and lining the inside of the medullary cavity at some distance from, the bone injury, there are bridges of osteoid trabeculæ which do not extend to the edge of the wound. The osteoblasts which line the trabeculæ appear smaller than usual. Their nuclei are smaller and more deeply stained than is usually the case, and there appears to be more protoplasm. Their arrangement is not quite identical with those in simple transverse fractures.

There seems to be no effort at absorption of the loose fragments, as there are no phagocytes present, but, instead, osteoblasts surround them as if they would be used as a framework for the formation of new bone. The medulla is largely replaced by young connective and granulation tissue. The bone defect is filled entirely with granulation tissue, there being no osteoid trabeculæ present.

Experiment 9. Operation performed August 7, 1905. Animal killed August 15, 1905. Fracture of eight days' duration.

Macroscopic Description. In bandaging the leg the foot had been left out and became the seat of moist gangrene, with sloughing. This did not extend underneath the dressing. There was no callus present. The fracture was found to have been greenstick.

Microscopic Description. The fracture of one of the bones is greenstick and the other complete transverse, with no displacement of the fragments. There is practically no infiltration of the muscles with exudate or connective tissue, and no evidence of fibrin in clot except in a couple of places between the ends of the fragments.

The periosteum is decidedly thickened for some distance on both sides of the line of fracture. Under it are osteoid trabeculæ in the form of a spindle with the thickest portion opposite to the line of fracture. There is less callus present in the greenstick fracture than in the complete one. Opposite to the line of fracture is found cartilage in the process of formation and in the stage of transition into osteoid tissue. In places the cartilage cells are extremely large and in close proximity to the small remnants of fibrin remaining between the ends of the bone (Fig. 3). Cartilage is found nowhere except opposite to the line of fracture. Most of the cartilage cells take a brownish stain and the ground substance blue.

The endosteum exhibits marked proliferation and more osteoid tissue than the periosteum. The osteoid tissue in the medullary cavity also assumes a spindle shape with the thickest portion opposite to the line of fracture. That there could never have been much displacement is shown by the fact that there is a narrow strip of medulla between the two medullary spindles on the two sides of the medullary cavity, as seen in section. There is also in the medullary canal, opposite the line of fracture, a very clear deposit of cartilage in the completely fractured bone and a minute deposit in the medullary cavity of the bone with a greenstick fracture at the point where there had been the greatest amount of separation. A fragment of bone lying loose in the medullary cavity is assisting in the formation of callus, having on its surface osteoid trabeculæ and cartilage.

The medulla is hyperplastic, there being much tissue which is myxomatous in appearance, numerous marrow cells, and a few fat cells. Between the fragments there is nothing but a small amount of fibrin, and, in places, a little connective tissue, the fragments being literally held in place by the two osteoid splints.

Experiment 10. Operation performed August 26, 1905. Animal killed September 4, 1905. Fracture of nine days' duration.

Macroscopic Description. There was a small amount of callus present and the ends of the bone were bound together, but there was some rocking motion of the distal ends of the fragments owing to the weakness of the callus. There was no mobility between the ends of the bone.

Microscopic Description. There is not a great amount of displacement of the ends of the fragments, but there is no union whatever between the apposed surfaces of the cortex. There appears to be a space between which is filled with detritus and some fibrin at the sides. The fragments are held in place by a very large amount of fibrocartilage, which extends from one fragment to the other, entirely enclosing the space before mentioned. In places this cartilage blends directly with the filaments of fibrin lining the cavity. In these situations it appears as if cells invaded the fibrin, which was then broken up into strands which were undergoing hyaline change. This is merely suggestive, and not convincing in appearance.

On the periosteal aspect of the fragments there is an abundance of osteoid trabeculæ covered by very large osteoblasts. The spaces between the trabeculæ take varying directions, but mostly obliquely from the site of fracture or almost parallel with the old cortex. The cartilage is present mostly at the site of fracture, forming a large, bulging mass at that area. Here it shows stages of transition into trabeculæ and fibrous tissue. The cells are very large and the ground substance between them becomes more prominent and takes a deeper eosin staining until gradually it encroaches on the cells and trabeculæ are formed.

In the medullary cavity there is a moderate amount of osteoid trabeculæ formation. It does not completely fill the canal, but goes up along the sides in contact with the cortex. The medulla has a hyperplastic increase of the connective tissue, with a decrease in the number of fat cells. Above this area the marrow is red and contains an abundance of very large cells, apparently with single, very large nuclei and an abundance of protoplasm. The cortex shows little or no absorption. Osteoid trabeculæ are seen forming on the surface of a loose fragment of bone.

Experiment 11. Operation performed May 19, 1905. Animal killed May 29, 1905. Fracture of ten days' duration.

This experiment consisted of a partial fracture with bone forceps.

Macroscopic Description. There was an organizing blood clot at the site of the operative wound. On the radius there was a nodule of callus about one-quarter of an inch long.

Microscopic Description. There is a great abundance of connective tissue all around the site of operation. On the periosteal surface there is a large amount of osteoid tissue which appears to take origin from osteoblasts and cartilage. This is found at some distance from the site of injury in the form of large, oval cells, with small, round, pale nuclei, the whole being surrounded by a wide halo and then a small amount of reddish-blue groundwork. In areas the ground substance increases, the cells decrease in size, and osteoid trabeculæ are formed. There is some fibrin and even free blood present.

The medulla is of the red variety, with a great incease in the connective tissue. In the medullary canal there is also some osteoid trabeculæ formation. The loose fragments do not show any proliferation on their surfaces, but, instead, show little pits or depressions in which are seen some large, brown cells, which do not stain well and are probably osteoclasts.

Experiment 12. Operation performed August 16, 1905. Animal killed August 28, 1905. Fracture of twelve days' duration.

Macroscopic Description. There appeared to be perfectly solid union of the fragments and there was very little callus.

Microscopic Description. There is a fair amount of osteoid callus, which is present in greater abundance on the periosteal side than on the medullary side of the bone. The callus is in the form of a spindle, tapering above and below the line of fracture. The position of the fragments is very good, there being no lateral or angular displacement, merely lengthening with consequent space between the ends of the fragments. In the periosteal callus the trabeculæ seem to be disposed in a longitudinal direction in the line of the bone so that the spaces between them run in a similar direction.

In the periosteal callus, surrounding the bone like a ring at the line of fracture, is a mass of cartilage with very large cells taking intense blue staining, with a blue, homogeneous, intercellular substance, and a condensation of the outer edge of the cells in the form of a capsule. On the edge of this mass of cartilage can be seen stages of transition into osteoid trabeculæ and what also appear to be transition stages into medullary cells. In areas directly next to and lying on the cartilage, are large multinuclear cells with deep-brown pigment, the exact significance of which it is difficult to determine; that is, whether they are derived from the cartilage (since some of the single cartilage cells in the same region have a brownish tint and appear as if the capsule were disappearing), or they are acting as phagocytes to absorb the ground substance of the cartilage and leave the cells as medullary cells. These large polynuclear cells are abundant throughout all the medullary spaces of the callus. The periosteal osteoid trabeculæ are very thin or absent at the line of fracture, the union being by cartilage only.

There are osteoid trabeculæ in the medullary cavity also, but not in as great abundance as on the periosteal side of the bone. There is a narrow tube of hyperplastic, vascular medullary tissue between the endosteal callus of the two sides of the bone, connecting the medullary canal above and below the line of fracture. There is a small area of cartilage in the endosteal callus which is not continuous over the line of fracture with that in the periosteal callus. Between the ends of the fragments there are no osteoid trabeculæ, but dense fibrous tissue and some cartilage (Fig. 4). The trabeculæ are very vascular. The cortex shows great porosity and absorption with an abundance of osteoclasts and some osteoblasts, but little or no bone formation.

Experiment 13. Operation performed July 27, 1905. Animal killed August 9, 1905. Fracture of thirteen days' duration.

Macroscopic Description. There was considerable mobility found at the site of fracture. Apparently not much callus had been thrown out. On removing the skin which was adherent to the site of fracture, considerable ecchymosis was found.

Microscopic Description. The fragments are not in apposition, there being considerable displacement of them. There is no union apparent. On the periosteal side there is but little osteoid callus formation. That which is present is very porous in nature and apparently undergoing absorption, as there are abundant osteoclasts present in the spaces between the trabeculæ.

On the endosteal side of the cortex there are but very few osteoid trabeculæ, and they are near the ends of the fragments. Between the fragments there are some loose fragments of bone, an abundance of fibrous tissue, and some fibrocartilage. The trabeculæ do not appear to be covered with osteoblasts to the normal extent. The medulla is rather fibrous and not very vascular. The cortex shows marked absorption by osteoclasts, being quite porous in the region of the fracture, with numerous osteoclasts throughout the widened spaces.

Experiment 14. Operation performed August 31, 1905. Animal killed September 14, 1905. Fracture of fourteen days' duration.

Macroscopic Description. Union was fair, but a slight amount of mobility could be obtained.

Microscopic Description. There is great displacement of the fragments and splintering of their ends. They are bound together by a large mass of fibrocartilage and connective tissue, the ends being free in some sections and embedded in the cartilage mass in others. On the periosteal aspect of the fragments there is considerable growth of osteoid trabeculæ. The spaces between the trabeculæ are filled with an abundance of osteoblasts and also contain osteoclasts. They do not appear, as yet, to be vascularized. In the medullary cavity there is a moderate growth of osteoid trabeculæ along the sides, but not filling the entire canal. The medulla is hyperplastic to a great extent and, near the articular ends of the fragments, is red, containing large cells with large, single nuclei. Some of them appear to be dividing.

At the line of fracture there are remnants of fibrin which appear to be broken up into strands by the invasion of connective tissue, etc. There are varying amounts of fibrocartilage present in the line of fracture, showing formation of trabeculæ and the action of the phagocytes in forming the canals between them. The loose fragments of bone exhibit absorption on one surface and the formation of trabeculæ on the other. These trabeculæ are undergoing absorption. In the cortex there is moderate osteoclastic absorption. The muscular fasciculi are separated by connective tissue.

Experiment 15. Operation performed August 17, 1905. Animal killed September 4, 1905. Fracture of eighteen days' duration.

Macroscopic Description. Union perfect, there being a fair amount of callus present, and no mobility of the fragments.

Microscopic Description. There is some displacement of the fragments, which are embedded in a mass of fibrous tissue and fibrocartilage. On the periosteal aspect there is a liberal amount of osteoid trabeculæ which blends with the cartilage in the region of the fracture. Osteoclasts are found throughout the trabeculæ and in the connective tissue between the fragments. There are trabeculæ on a loose fragment. There is absorption of the cortex with a lining of osteoblasts in the widened Haversian spaces.

The medulla is hyperplastic and, near the articular ends, red, with very large cells having a single nucleus with a tendency to divide. There are some osteoid trabeculæ in the medullary cavity. Across the line of fracture are connective tissue and cartilage.

Experiment 16. Operation performed August 7, 1905. Animal killed August 28, 1905. Fracture of twenty-one days' duration.

Macroscopic Description. There was perfectly solid union and the site of the fracture could not be found. The leg was dissected and a small, dark spot found which was probably the site of a greenstick fracture.

Microscopic Description. The specimen was most likely a greenstick fracture with perfect union, the site of fracture being recognizable by the greater thickening of the bone at one place, combined with increased vascularity and hyperplastic condition of the medulla, and the porosity of the bone at that situation.

It is almost impossible to distinguish callus from old bone, since whatever callus there has been is as dense as the original bone. In the medulla of the widened spaces of the cortex and in the medulla of the main medullary canal, where it is in contact with the bone (probably new), are a great number of polynuclear, brown-staining, pigmented cells (osteoclasts), some lying in pits or depressions. Lining the cavities are polygonal cells, probably osteoblasts. All the Haversian spaces on the side of the fracture are widened and are lined with osteoblasts. They contain bloodvessels or numerous spindle cells and some small, round, eosinophilic cells similar to bone-marrow cells. The marrow is hyperplastic and of the red variety.

Experiment 17. Operation performed July 28, 1905. Animal killed August 21, 1905. Fracture of twenty-four days' duration.

Macroscopic Description. The fracture was found to have been perfectly united. The ends were apparently not in close apposition and projected in places. There was not an excessive amount of callus.

Microscopic Description. There is a fairly abundant osteoid callus, especially in the periosteum. It is impossible to determine the extent of the endosteal callus, because, owing to the angular displacement of the fragments, the sections do not go through the specimen in the proper plane to show the condition of the medullary cavity in the region of the fracture. The osteoid trabeculæ appear to be in the stage of absorption, there being quite a few osteoclasts applied to their surfaces. They are also lined with osteoblasts on them. There do not appear to be any new layers of bone.

Between the fragments there are no osteoid trabeculæ, but dense, cellular connective tissue, with some cartilage containing lime salts in places. The union is apparently fibrous. There is extensive rarefaction of the bone, the widened Haversian spaces being covered, in areas, with large, multinuclear osteoclasts taking a brown stain. Some of the spaces contain bloodvessels and others merely a cellular connective tissue. In some there are apparently osteoblasts.

Experiment 18. Operation performed July 31, 1905. Animal killed September 4, 1905. Fracture of thirty-five days' duration.

Macroscopic Description. The fracture had united perfectly, with the formation of a very large callus. There was no mobility present.

Microscopic Description. There is a very large mass of callus, but the relations of the fragments cannot be determined because of great displacement. The callus is, for the greater part, fibrocartilage showing distinct stages of transition into osteoid tissue. There does not appear to be a great amount of absorption, as osteoclasts are not present in great numbers except in and around a couple of detached fragments. In portions of these fragments there is distinct lacunar absorption and in other areas a deposition of layers of new bone.

There are practically no signs of absorption of the cortex. The Haversian spaces are not dilated and there are no newly formed spaces. There does not appear to be a great amount of endosteal bone formation, most of the callus being periosteal in origin. There is a considerable quantity of osteoid tissue, especially in the portions of the callus at the greatest distance from the line of fracture. The marrow is red and hyperplastic, containing in areas abundant myxomatous-looking cells.

SERIES 2.

The operations of this series, performed upon dogs, consisted in making subcutaneous fractures of the tibia and fibula by forcibly bending them over the side of the table. The legs were placed in shaped wooden splints which were retained in position by a sodium-silicate dressing.

Experiment 19. Operation performed December 6, 1905. Animal died December 27, 1905. Fracture of twenty-one days' duration. The animal died apparently as the result of infection from an ulcer upon the abdomen caused by the bandage.

Macroscopic Description. There was no displacement, but some mobility of the fragments. A small amount of callus was present. The fibula appeared to be slightly thickened opposite to the site of fracture, but perfectly sound.

Longitudinal section through the bone revealed a very good apposition of the fragments, with only a small amount of space between the ends. There was very little periosteal callus present, but there was a thin layer of new white bone 2 mm. thick on the internal angle formed by the fragments, extending 15 mm. below and 10 mm. above the line of fracture. It was much thinner on the anterior surface of the bone. Directly opposite to the line of fracture there was no new bone, but cartilage. The medullary cavity was filled for 2 cm. above and 2.5 cm. below the line of fracture with young, reddish-yellow, very vascular, spongy bone, except for the distance of 5 mm. above and below the line of fracture, where it was cartilaginous in appearance. The nutrient artery penetrated the upper plug of bone. The line of fracture on the anterior aspect of the bone was filled with cartilage.

Microscopic Description. Between the ends of the bone nothing but dense fibrous tissue can be seen. In places, blood and fibrin intervene between this tissue and the bone. This dense fibrous tissue traverses the entire line of fracture across the medullary canal. On each side of the tissue there is a layer of fibrocartilage entirely across the line of fracture and in the periosteal callus. On the periosteal aspect of the bone there is a moderate proliferation of osteoid trabeculæ, more pronounced in places where there are inequalities at the site of fracture. The increased amount of trabeculæ fills in the depressions made by those inequalities. The trabeculæ are no longer covered with osteoblasts in layers, but there is some absorption of the periosteal trabeculæ by osteoclasts. In the spaces between the trabeculæ there are loose connective tissue and thin-walled bloodvessels. There is considerable absorption of the cortex in some areas and none in others. These places contain numerous osteoclasts, but no osteoblasts. The ends of the fragments are undergoing absorption.

The medullary cavity is filled, to a considerable extent, with loose osteoid trabeculæ, which extend flush with the ends of the fragments, where they abut against fibrocartilage. The spaces between the trabeculæ are large and contain loose connective tissue, marrow cells, and leukocytes (polymorphonuclear). Almost all the spaces contain large, thin-walled bloodvessels engorged with blood. There is practically no absorption by osteoclasts. The connective tissue of the marrow is considerably increased in amount, but not the marrow cells.

Experiment 20. Operation performed July 3, 1905. Animal killed August 7, 1905. Fracture of thirty-five days' duration.

This operation was performed with a saw through an incision.

Macroscopic Description. There was a very small drop of pus in one stitch-hole. The deeper portions of the wound were not at all infected. There was a very large callus extending for about 12 mm. on each side of the bone wound, and projecting from the bone for about 12 mm. The union of the fragments was very firm.

Longitudinal section through the bone revealed the fact that the fracture had been made where the cancellous portion of the bone joins with the compact portion. There was perfect union of the fragments, the entire medullary cavity for 15 mm. being completely filled in with newly formed cancellous tissue, containing in areas (Fig. 5) small islands of cartilaginous tisue. The callus, on the outer aspect of the bone, extended from 2.5 to 3.5 cm. toward the epiphysis and, in places, was 6 mm. thick. The portions of the callus nearest the epiphyses and next to the old cortical bone had gone on to the formation of compact bone, being more compact the farther removed from the site of fracture. Just opposite the line of fracture, where the callus was thickest, it existed still in the form of cartilage. The bone union was mostly cartilaginous, showing that ossification proceeds from the point at which the periosteum and endosteum remain attached to the cortex.

Microscopic Description. There is some lateral displacement of the fragments. There is no actual bony union between the ends of the fragments, but a thin layer of very dense fibrous tissue, which is continuous, on the periosteal side of the old callus, with fibrocartilage.

Osteoid callus is present in considerable amount on the periosteal aspect of the bone, tapering from the line of fracture upward and downward on the fragments. In areas this tissue is dense and in other areas quite rarefied (that is, where it is abundant to fill in a defect caused by the displacement of the fragments, it is dense externally, between the end of one fragment and the side of the other, and rarefied in the deeper portions, where it is opposite the medullary cavity of the other fragment). Osteoclasts are abundant. Osteoblasts line the medullary and Haversian spaces.

The medullary canal contains a moderate amount of callus which is quite rarefied, especially in its centre. Osteoclasts and osteoblasts are present side by side. This is additional proof that the process of repair is only in progress and not complete. The marrow is red.

There is great absorption of the cortex, so that the ends, in places, appear as mere spicules. The Haversian spaces contain osteoclasts as well as osteoblasts. There does not appear to be any hyaline cartilage present, and only a moderate amount of fibrocartilage. The remainder of the unossified tissue is dense and fibrous.

Experiment 21. Operation performed October 31, 1905. Animal died December 27, 1905. Fracture of fifty-seven days' duration.

Macroscopic Description. There was firm union of both bones, with a large callus about 25 mm. long by 12 mm. thick on the tibia and 18 mm. by 12 mm. on the fibula. There was angularity of the fragments anteriorly.

On longitudinal section through the bone, it was found that there was some displacement of the upper fragment forward on the lower fragment, so that the posterior side of the cortex of the upper fragment plugged the medullary cavity of the lower fragment. The angle on the posterior aspect caused by the projection forward of both fragments was completely filled in on the periosteal side, for a distance of 25 mm. on each fragment, with new bone and cartilage 10 mm. thick opposite the line of fracture and arranged as follows: Quite dense bone farthest removed from the site of fracture; then brownish, porous bone against the cortex to the line of fracture, where it was replaced by cartilage. This cartilage assumed the form of a bracket with its point toward the bone and its base outward (Fig. 6), being 15 mm. at its longest point. On the anterior aspect of the angle, on the periosteal side, there was practically no callus excepting a small amount of new bone 7 mm. in extent filling the space formed by the projection of the upper fragment. The thickness of the callus almost reëstablished the straight line between the knee and the ankle. The medulla of the upper fragment was filled for 20 mm. and the lower for 5 mm. with white, spongy bone, beyond which was mottled red or yellow marrow. A thin layer of cartilage intervened between the ends of the fragments. The posterior aspect of the cortex of both fragments near the thick callus had a porous appearance, as if some absorption had taken place.

Microscopic Description. There is great angularity of the fragments. The microscopic findings confirm the gross findings. Abundant, rather dense osteoid tissue is found in the angle between the fragments. An apparently younger formation, with larger spaces than in the deeper portion, is seen on the surface. The general direction of the medullary spaces in the new bone is almost vertical to the cortex. There is some absorption of the deeper layers, with the formation of longitudinal canals (Fig. 8).

On the outer side of the angle there is very little osteoid bone formation and this does not extend to the ends of the fragments.

In the medulla one fragment is filled for some distance with quite compact osteoid tissue. This is where the outer side of the cortex of one fragment abuts against the medullary surface of the other fragment. In the medullary canal of the other fragment, where the inner side of the cortex of the previously mentioned fragment abuts against it, there is medullary osteoid tissue undergoing absorption, but not present in great abundance.

There is hardly any absorption of the cortex on the outer side of the bone away from the angle of displacement, while the cortex on the inner side next to the extensive callus has undergone considerable rarefaction with a lining of osteoblasts, bloodvessels etc., in its dilated spaces. At the line of fracture there is fibrocartilage all the way across, especially marked in the angle between the fragments where it widens out into a wedge shape. Between the fragments it assumes more the character of very dense fibrous tissue than fibrocartilage. It is quite vascular in areas. One portion of the cortex shows no attachment to it whatever, but was evidently separated by a space. On the edges of the mass of cartilage there are evidences of advancing bone formation, the cartilage being penetrated by bloodvessels, osteoblasts (?), and marrow cells, accompanied by a deposition of calcium salts at the edge and the formation of new trabeculæ (Fig. 10). This process is well illustrated in the fibula, which presents phenomena similar to those described above. Most of the medulla is red and contains some osteoclasts.

Experiment 22. Operation performed December 8, 1905. Animal killed February 9, 1906. Fracture of sixty-three days' duration.

Macroscopic Description. The leg was perfectly united, only a very small, spindle-shaped callus being present. Longitudinal section (Fig. 7) through the medulla revealed perfect union of the fragments, which were in rather good position, there being only slight angular deformity forward, associated with slight forward dislocation of the lower fragment on the upper. The medullary cavity was continuous for a part of the distance. There was quite an abundant periosteal callus on the posterior side of the bone, extending upward on the cortex 22 mm. and downward on the lower fragment 15 mm., being 5 mm. thick at its thickest portion directly opposite to the line of fracture. The callus here consisted of firm white bone at the portions farthest removed from the line of fracture, so dense as to be almost indistinguishable from the old bone. As it approached to within 10 mm. of the line of fracture it became more cancellous in structure and gradually darker in color until, directly opposite it, there was a wedge-shaped mass of cartilage with its base, measuring 5 mm., outward. On the periosteal side of the anterior aspect of the bone there was practically no callus excepting filling in the projection caused by the protrusion forward of the lower fragment. Here the newly formed bone was flush with the surface of the lower fragment and extended on the upper fragment 15 mm. In the medulla there was a small amount of new spongy bone on the sides of the cavity for 10 mm. on the lower and 5 mm. on the upper fragment. The entire line of fracture consisted of cartilage

Microscopic Description. Microscopic sections confirm the macroscopic description to a great extent. There was no bony union between the two fragments. There is dense fibrous tissue extending along the line of fracture opposite to the medullary canal. This contains leeshes of bloodvessels. At the internal angle between the two fragments there is a wedge of fibrocartilage which is continuous at the edge with osteoid trabeculæ. These are very thick at the internal angle and continue the general line of the bone. The periosteal trabeculæ are densest on the surface and have undergone great absorption, with the formation of widened spaces in the lower level of the callus. These spaces are filled with loose connective tissue, bloodvessels, small round cells, and osteoclasts. Where it borders on the cartilage there are especially large numbers of osteoclasts and in some places the cartilage seems to be affected by them. In places it looks as if the cartilage were first eaten out by the osteoclasts, followed later by a deposit of lime salts at the margins of the cartilaginous trabeculæ. In other places it looks as if the cartilage were first penetrated by bloodvessels. There is considerable absorption of the cortex. Like the trabeculæ of the new callus, its spaces do not appear to contain active osteoblasts, but are lined by flat cells.

There is only a small amount of osteoid tissue in the medullary cavity. The trabeculæ look as if they had been dense, but had suffered great absorption by osteoclasts. The medulla is congested with red blood corpuscles, but is not particularly cellular. There are no osteoclasts except in apposition with bone. There is a goodly number of leukocytes where there is absorption going on, so that they may have some connection with that process.

Experiment 23. Operation performed November 21, 1905. Animal died February 14, 1906. Fracture of eighty-five days' duration.

Macroscopic Description. The leg was found to be perfectly sound, having united with practically no displacement as far as could be determined without section through the bone. There was a moderate amount of firm callus present, extending 15 mm. on each side of the line of fracture. The callus on the anterior aspect was very red and vascular-looking; that on the posteior aspect, projecting about 7 mm., was deeply grooved by one tendon in particular, which had to be dug out of it. It was more lightly grooved by several others.

On longitudinal section of the bone it was found that there had been slight backward displacement of the upper fragment on the lower, with the formation of quite abundant periosteal callus, especially on the posterior aspect. This posterior callus was composed mostly of brownish, cancellous bone, with the exception of the portion nearest the old bone. Just below where the upper fragment ended it appeared as if condensation had taken place in order to take up the strain from the upper fragment. It contained very little cartilage. The periosteal callus on the anterior aspect contained a wedge of cartilage with a base of about 2 mm. and with its apex at the line of fracture. A thin layer of cartilage extended across the entire line of fracture. The periosteal callus on the anterior aspect contained more compact bone than that on the posterior aspect.

The medullary cavity was plugged with cancellous bone for about 15 mm. on each side of the fracture, beyond which the marrow was red in color.

Microscopic Description. The position of the fragments is good and there is abundant callus. Across the line of fracture there are intermingled islands of cartilage and osteoid trabeculæ, which are continuous with the osteoid trabeculæ in the medullary canal. On the periosteal aspect there is more cartilage to be seen, with a tendency to wedge formation. The most exterior portion of the callus next to the periosteum consists of a complete bridge of wide, osteoid trabeculæ, continuous from one fragment to the other. These spaces and the trabeculæ next to the cartilage are lined with osteoblasts. Most of the osteoid trabeculæ are quite dense, but there is some absorption by osteoclasts. This is especially marked in its deepest portion next to the old cortex, where it is forming an Haversian canal. The cortex has undergone some absorption, but the canals are not lined with osteoblasts.

The medulla is plugged with osteoid trabeculæ, on which are osteoblasts in some areas and not in others. There is little, if any, absorption of the medullary trabeculæ. The marrow is mostly of the hyperplastic variety, with an increase of connective tissue and some few cells. There are quite a number of polymorphonuclear leukocytes in other areas.

Experiment 24. Operation performed November 3, 1905. Animal killed February 9, 1906. Fracture of ninety-eight days' duration.

Macroscopic Description. The fracture was united, but the union was not bony. There was quite an abundance of callus present and some internal bowing at the site of fracture, with very slight rocking mobility.

On longitudinal section through the bone, anterior angularity of the fragments, with the formation of a very large periosteal callus on the posterior aspect, was found. This callus extended for 25 mm. on both fragments and was 10 mm. thick at its thickest part, opposite to the line of fracture. It was composed of dense, white bone; brownish, cancellous bone and cartilage, arranged as follows: The dense, white bone was farthest from the site of fracture for 8 mm., where it left the bone and continued on the outside of the callus as a layer, 3.5 mm. thick, to the edge of the cartilage, which was present as a wedge-shaped mass with its base (13 mm.) outward and its apex tapering to the line of fracture. The intervening space between the outer shell of hard bone, the cortex, and the cartilage was filled with cancellous bone. On the anterior aspect of the periosteal surface of the bone there was a small amount of callus consisting of a wedge of cartilage with its base (10 mm.) outward (4 mm. thick) and a tapering shell of white, dense bone 8 mm. long by 2 mm. at its thickest portion. The medullary cavity was completely closed for a distance of 10 mm. on each side of the line of fracture with spongy, brownish-white bone, which then gradually crept up the sides, fine trabeculæ being felt on the anterior aspect of the upper fragment 25 mm., and of the lower fragment 20 mm., from the site of fracture. They did not extend quite so far on the posterior aspect of the medullary cavity. There was a streak of cartilage, 2 mm. thick, extending across the medulla at the site of fracture. Beyond the new medullary bone there was red marrow for about 8 mm. and then yellow marrow. The nutrient artery pierced the upper medullary new bone.

Microscopic Description. Sections confirm macroscopic description with reference to osteoid tissue, position of fragments, etc. On the periosteal aspect of one side of the bone there is a great abundance of osteoid callus on each fragment, separated by a wedge of dense fibrocartilage. The general trend of the canals in the osteoid tissue is almost vertical to the old cortex and the bone is very dense, excepting that in the lower levels of these masses there is great absorption (as described macroscopically) forming very wide canals parallel to the cortex. These canals contain loose connective tissue, osteoclasts, bloodvessels, and leukocytes. The outer layer remains dense, there being no absorption whatever. The fibrocartilage does not show much transition, but seems to have been separated from the neighboring tissue, leaving a space which is filled with a diffuse, slightly granular, eosin staining material. This extends almost entirely across the fragments.

On the other side of the fracture there is only a small amount of periosteal callus, which also abuts against a fibrocartilage wedge. Here, likewise, the osteoid tissue is dense, but instead of having undergone absorption in its deeper portions, more absorption has taken place in its superficial areas. Some of the spaces of the periosteal callus which have been caused by absorption are lined with apparent osteoblasts somewhat flattened out and not active.

The cortex has suffered great rarefaction by osteoclasts. The surface and ends of the fragments have undergone some absorption. The medullary cavity is partially filled with osteoid trabeculæ, which show considerable osteoclastic absorption. They do not appear to be covered with osteoblasts. The marrow is red in some places and contains some osteoclasts. There are no osteoid trabeculæ between the ends of the cortical portions of the fragments, but there appears to be almost the formation of a false joint, with very slight, if any, motion.

Experiment 25. Operation performed November 7, 1905. Animal died March 16, 1906. Fracture of one hundred and twenty-nine days' duration.

Macroscopic Description. Perfect union of the fracture was found with the formation of a very small callus which was slightly grooved for a couple of tendons. There was practically no displacement of the fragments as far as could be determined.

Longitudinal section through the bone demonstrated that the reposition of the fragments had been almost perfect, there being no displacement whatever. The line of fracture was distinctly visible and was completely filled with white, rather dense bone. On the periosteal side, extending for about 5 mm. on each side of the fracture, and for about 1 mm. in thickness, there was a layer of white, hard, new bone. There was no cartilage or dark, cancellous bone to be seen on the periosteal side of the bone. The medulla contained a very small area of very porous, reddish bone, apparently in the process of absorption, not extending entirely across the medullary cavity, and about 4 mm. in length. Running through its centre was the large nutrient artery. There was very slight forward angularity of the fragments.

Microscopic Description. The fragments are in perfect alignment, there being slight separation of the two ends on the one side. On both sides there is a small amount of periosteal osteoid callus which shows the general direction of the old spaces to be almost vertical, but there had been great absorption of the osteoid tissue with the formation of very large longitudinal spaces (Fig. 9). These spaces, in turn, are lined with new, dense bone, which takes brilliant eosin staining in contrast with the bluish stain of osteoid tissue. In addition, there is a striation parallel with the sides of the cavity, viz., formation of lamellæ. The cells are spindle shaped, as in old cortical bone. The periosteal osteoid tissue also has undergone considerable lacunar absorption on the surface. The cells, having been greatly destroyed or shrunken, it cannot be determined whether or not the spaces are lined with osteoblasts. The medullary cavity contains some scattered trabeculæ in the region of the fracture. Here, and to some extent up the sides of the bone, the marrow is red, but yellow elsewhere. There is great absorption of the cortex, especially at the line of fracture, where, in some sections, can be noted very large spaces which are beginning to show a deposit of new bone. On one site there have been formed distinct communications between canals of the cortex of both fragments, and the union is complete.

Experiment 26. Operation performed November 21, 1905. Animal killed August 10, 1906. Fracture of two hundred and sixty-two days' duration.

Macroscopic Description. The animal was killed after having been in the country for several months in order to keep its health up to a high standard, owing to the fact that long fractures could not otherwise be obtained since the animals died because of long confinement.

The leg was perfectly united and firm. There was a small-sized callus at the site of the fracture, but the alignment of the bone was very good. On removing the skin the callus was found to be grooved by the tendons passing over it. The length of the callus on the external surface was not over 12 mm.

On longitudinal section through the bone the position of the fragments was found to be very good. The line of fracture was still visible as a whitish line through the callus. At the line of fracture, in the small external callus and extending part way between the fragments, was a small area of cartilage. The remainder of the external callus was rather hard bone. The medullary cavity was completely filled with vascular-looking, cancellous bone continuous with the normal cancellous portion of the lower ends of the bone and extending above the line of fracture 2 cm. The nutrient artery entered the centre of it. Above the cancellous plug was deep-red marrow. The plug of new bone was denser at the line of fracture than elsewhere. The ends of the fragments appeared somewhat darker than normal, looking as if there had been some absorption and it was intimately blended with the interposed callus.

Microscopic Description. There was almost perfect apposition of the fragments with a moderate external callus of osteoid tissue and a liberal plug of medullary osteoid tissue. In the external callus there are still remains of a wedge-shaped piece of cartilage or dense connective tissue, with evidences of its having extended across the entire line of fracture. In the specimens this is now a very small wedge on one side and is almost completely absent on the other side. The remainder of the periosteal callus shows superficial lacunar absorption and absorption of the deeper portions with the formation of medullary spaces parallel to the old cortex. The medullary cavity is filled partly with cancellous osteoid tissue which has suffered great absorption except near the line of fracture, where it is denser. The trabeculæ are thin and wide apart. The spaces are filled with red marrow which shows numerous osteoclasts. Some of the spaces contain fat cells also. There is great absorption of the cortex near the ends. At the line of fracture, where the two portions of the old cortex abut against each other, there are evidences, owing to the bluish character of the stain, of there having been originally fibrous tissue between the bones. This material is now replaced by osteoid trabeculæ containing more numerous and smaller spaces between them than other portions of the bone. These spaces unite with those of the old cortex, and the bone is practically healed. The spaces are lined by osteoblasts. The trabeculæ take a bluish-red stain.

Experiment 27. Operation performed November 7, 1905. Animal killed August 14, 1906. Fracture of two hundred and eighty days' duration.

This animal had also been in the country for several months.

Macroscopic Description. The leg was found to have apparently united very solidly, the animal having used it perfectly and there having been no mobility of the fragments. There was some nodulation felt at the site of fracture. On removing the skin there was found some callus extending up about one-half inch on each fragment, which was deeply grooved by the tendons that passed over it. The callus bound the tibia and the fibula tightly together.

On longitudinal section the periosteal callus was found to extend up on each fragment about 2 cm. and was about 1 cm. thick. It was composed almost entirely of a dark, vascular, porous, spongy bone divided in half at the line of fracture by a thin line of cartilage. This line of cartilage extended all the way across the medullary cavity. The medullary cavity was plugged completely for about 1 cm. on each side of the line of fracture with dense, white bone, beyond which (for about 12 mm.) there was porous vascular bone; beyond this, red marrow.

Microscopic Description. The fragments are in good position and there is abundant callus. On the periosteal side of the fracture there is a large, spindle-shaped callus showing thick trabeculæ with large spaces between them. These spaces are filled with marrow containing fat cells and marrow cells. The trabeculæ are dense in construction and take no particular direction, being very much like normal cancellous bone. The superficial surface shows considerable osteoclastic absorption.

In the medullary cavity there are similar trabeculæ for some distance on each side of the line of fracture. The trabeculæ are not so thick and the spaces between them are slightly larger than in the periosteal callus. There is considerable absorption of the cortex at the ends and in its substance generally, the widened spaces communicating freely with those of the periosteal and medullary trabecular spaces. The marrow is not present in great abundance and is more cellular than normal, but not red.

At the line of fracture, extending across the entire mass, is a moderately thin layer of dense fibrous tissue and fibrocartilage. In areas this shows transition into osteoid trabeculæ. In other areas it has a diffuse hyaline appearance, taking the eosin stain. There is no angular displacement and no wedge of cartilage, merely a thin strip in the periosteal callus.

SERIES 3.

This series, performed upon dogs, had for its object the determination of the activities of the various portions of the bone independent of extrinsic forces. Each operation consisted in making an incision over the tibia to the bone and then making a sawcut half-way through the bone in order to injure all the elements of the bone as in fractures, but with none of the attendant displacement of the fragments, etc. The skin was then sutured and the leg placed in a shaped splint and wrapped with a sodium silicate bandage.

Experiment 28. Operation performed September 15, 1905. Animal killed September 20, 1905. Fracture of five days' duration.

Macroscopic Description. The wound appeared to have healed by first intention, but on considerable pressure there was an escape of a small drop of thin, yellow, serous fluid between the stitches. There were signs of ecchymosis around the wound and down the leg to the ankle. On removal of the skin, considerable ecchymosis was found in the region of the wound, but no infection was apparent. The sawcut appeared to be filled in with a gelatinous, pinkish-white material. There was no free, unorganized blood present.

Longitudinal section through the bone revealed the fact that the sawcut had been made almost directly opposite to the point of entrance of the nutrient artery into the medullary cavity. The sawcut was filled flush with the surface of the bone with a brownish, fairly firm material. The periosteum above the wound appeared to be a little thickened, but there was no callus. In the medullary cavity, almost completely filling its lumen, there was a white, glistening mass that appeared to be young callus, next to which was red bone-marrow. The remainder of the marrow at the lower end was yellow. Above the periosteum there was a blood clot.

Microscopic Description. The sawcut proved to have been made just at the junction of the cancellous and the compact bone. In making it, a sliver of bone was bent and forced into the medullary canal. The periosteum has not proliferated, but the tissues are infiltrated with blood. The sawcut is filled with blood clot and no formative cells appear to be present.

In the medulla at the site of the wound, and filling almost one-half the lumen of the canal, there is a very loose cellular connective tissue, much like myxomatous tissue. This appears to arise from the connective tissue between the fat cells, for at the outer edge of the mass it can be noted that fat cells are gradually decreasing in number and the intercellular substance increasing until the fat cells and the small, round, eosinophilic medullary cells become entirely obliterated.

Embedded in this mass of tissue is a broken or bent-off spicule of old bone, on the surface of which are large polygonal cells. New trabeculæ are apparently formed by these cells secreting a homogeneous matrix which is strongly eosinophilic and which finally includes some of the cells in the mass.

On the medullary surface of the cortex, the endosteum can be seen to thicken on each side of the sawcut and to form polygonal cells which also form trabeculæ in the same manner. The cortex in the neighborhood of the wound shows dilated Haversian spaces, but that these are normal, being the beginning of the formation of cancellous tissue and not due to absorption, is shown by the arrangement of the bone cells around their outskirts. These spaces also contain osteoblasts and trabeculæ. There is no absorption and no phagocytes are present. The marrow is slightly cellular.

Experiment 29. Operation performed September 28, 1905. Animal killed October 5, 1905. Fracture of seven days' duration.

Macroscopic Description. The wound had united perfectly, but was encrusted with a yellowish material like pus and a little pus came out of one stitch-hole on pressure. On removal of the skin, no pus was found in the deeper tissue. There was nothing covering the sawcut, which was partially filled with a whitish material. The periosteum was slightly thickened.

On longitudinal section, the periosteum was found to be thickened below and above (but not within 1 cm. above) the sawcut. The sawcut was one-fourth filled with a yellowish-white material which was not very hard. The medulla appeared to be condensed for about 2 cm. in the region of the sawcut, which had been made about 1 cm. below the point of entrance of the nutrient artery into the medullary cavity.

Microscopic Description. The sawcut is about half-filled with round cells the nature of which cannot be accurately determined, *i. e.,* whether they are leukocytes or marrow cells. The periosteum at some distance from the wound has increased slightly in thickness, with the formation of large, polygonal, formative cells in the lower layers. There is no evidence whatever of trabeculæ formation. There are no signs of cartilage. The superficial surface of the cortical bone has upon it pits or depressions, the result of phagocytosis by osteoclasts which are found lodged in them. There does not appear to be any widening of the Haversian spaces.

The marrow is of the red cellular variety and, in the region of the wound, the endosteum is thickened, with the formation of large polygonal osteoblasts. These are found extending to the centre of the medullary cavity opposite to the wound. Among these masses of cells can be seen a transition into osteoid trabeculæ by the secretion of a hyaline matrix, with a rearrangement of the cells so that they lie as a layer on the newly formed trabeculæ. They extend almost completely across the wound in the form of an arch. A few separate fragments in the medulla are being absorbed by osteoclasts. There are no trabeculæ in the sawcut and no absorption of the sides of the cut. Large multinuclear cells are present in the medulla.

Experiment 30. Operation performed September 8, 1905. Animal killed September 16, 1905. Fracture of eight days' duration.

Macroscopic Description. The wound was found to have united by first intention. On removing the skin practically no callus could be seen, but the sawcut was filled with a reddish, gelatinous material.

On longitudinal section through the bone, the periosteum was found to have proliferated very little if at all. On the medullary side of the sawcut here was a condensation of the medulla and, at one place, there was new bone formation projecting into the medullary cavity. The sawcut was on the opposite side of, and about 7 mm. lower than, the entrance of the nutrient artery into the medullary cavity.

Microscopic Description. Examination with the lower powers shows that in none of these sections had the sawcut penetrated quite into the medulla, that area probably being in the other half of the bone preserved for grinding. That the cut must have penetrated the medulla is demonstrated (?) by the fact that there is marked reaction in the medullary cavity in the region of the sawcut.

There is a small amount of fibrous thickening of the periosteum, but no proliferation of the osteogenetic or deeper layers is noticed excepting in small areas, where the smooth external surface of the cortical bone has been eroded by osteoclasts. Here there appears to be a reaction in the periosteum.

The endosteum does not show much reaction except in the region of the sawcut, where there is a new formation of osteoid tissue with its large cells containing large nuclei. The trabeculæ are covered with large polygonal osteoblasts. This new bone mass projects into the lumen of the medullary cavity about one-fourth the distance across the canal. The hyperplastic marrow in the same region shows numerous osteoclasts or polynuclear, brown-staining cells. The remainder of the medulla is of the red variety, with its eosinophilic, variously sized, brown, mononuclear cells. The bone shows practically no reaction, the spaces not being dilated and containing no proliferating cells.

Experiment 31. Operation performed July 24, 1905. Animal killed August 2, 1905. Fracture of nine days' duration.

Macroscopic Description. The wound had healed by first intention On removal of the skin, the wound in the bone was found not to have been entirely filled with callus.

Microscopic Description. The sections demonstrate that the sawcut had been made just where the cancellous joins the compact porton of the bone. There is no proliferation of the periosteum except to a very slight degree where the cortex appears to be eroded.

The endosteum does not appear to be proliferated excepting over the trabeculæ of the old cancellous bone. The medullary tissue is red on the margins and then, as one approaches the sawcut, becomes more admixed with young connective tissue. In the immediate neighborhood of the sawcut are delicate trabeculæ of osteoid tissue covered by osteoblasts. This osteoid tissue extends above and below the sawcut and half-way across the medullary cavity. It also extends upward into the sawcut for about half its depth, where it becomes mostly cellular connective tissue with spindle cells and some young, thin-walled bloodvessels. On the periosteal side of the wound, the sawcut is filled with fibrillated fibrin streaming out of the wound. There is no cartilage. There is slight absorption of the cortex present.

Experiment 32. Operation performed July 24, 1905. Animal killed August 3, 1905. Fracture of ten days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin, at the site of the bone wound there was found a very minute quantity of a reddish-yellow, turbulent fluid which appeared to be debris of blood clot, etc. At the site of the fracture there was a moderately firm, reddish-yellow accumulation of material for about 6 mm. in extent.

On longitudinal section it was found that the sawcut had entered the medullary cavity, which was filled with callus for over 6 mm. above and below the sawcut. The callus was rather firm. Owing to the muscle, etc., it was difficult to state how much callus was formed on the periosteal side.

Microscopic Description. There is some debris present with some slight inflammatory reaction but probably no marked infection. The wound is filled with dense granulation tissue with granular debris on the top. There is marked thickening of the periosteum with the formation of a fairly thick layer of osteoid tissue all around the bone excepting in the immediate neighborhood of the wound, where there is no callus. The periosteal process of bone formation is shown clearly. The periosteum is raised up. Both layers, the fibrous and the osteogenetic, are thickened. The osteogenetic layer is composed of large cells with large vesicular nuclei. These cells show the deposition, in parallel lines, of a homogeneous eosin staining material in the matrix between the cells. This material accumulates in greater bulk and includes some of the cells in its substance, while other cells are arranged in layers on the surface of the trabeculæ. Still others lose their polygonal shape and become more or less spindle shaped, forming the medullary tissue between the trabeculæ. Deeper down the trabeculæ are much thicker and the spaces between them much smaller, showing that they are older and demonstrating the action of the osteoblasts in their formation. The periosteum must have been subject to considerable chemical or toxic irritation to cause this profuse growth of osteoid tissue, for there was not much mechanical irritation present. The medullary cavity is filled across the entire lumen, and for about 12 mm. on each side of the wound, with loose osteoid trabeculæ, distributed in greater part, in irregular clumps, the outlines of which are defined by connective tissue or bloodvessels. In the medullary spaces between the trabeculæ are many fat cells. The osteoblasts are not arranged in many places, as usual, in layers, but are irregularly placed and blend directly with the trabeculæ. The gradual thickening or proliferation of the tissue between the fat cells, with the gradual deposition of osteoid tissue in this proliferated tissue, appears like cause and effect. The endosteum also shows thickening, with a deposition of trabeculæ on the inner aspect of the cortex, but not to a very great degree.

There is slight widening of the Haversian spaces of the cortex, but no lacunar absorption or proliferated tissue in the dilated spaces. There is lacunar absorption by osteoclasts of the sides of the sawcut (Fig. 12) and on the periosteal aspect of the cortex next to the sawcut. The cut itself is filled with granulations which are rather dense and vascular in the more superficial portions. In the deeper portions, toward the medulla, this granulation tissue is blended with the proliferated tissue of the medulla, from which it probably takes origin. It differs in that the cells are more spindle shaped, and more closely packed. Toward the medullary side of the sawcut, parallel with its edge and in the direction of the streams of granulation tissue, can be seen trabeculæ (Fig. 12) forming in the proliferated tissue. Large multinuclear cells are found in the marrow.

Experiment 33. Operation performed August 18, 1905. Animal killed August 29, 1905. Fracture of eleven days' duration.

Macroscopic Description. The wound had healed by first intention. There was a small amount of external callus at the site of the wound and the sawcut was somewhat filled in.

Microscopic Description. In the medullary cavity there is abundant callus in the form of rounded masses, separated almost opposite the sawcut by large bloodvessels around which there are no trabeculæ. They unite at the base of the sawcut and are continued into it almost to the surface, in the centre of a mass of cellular fibrous tissue. The trabeculæ enter the sawcut from the medullary cavity, forming vertical colums parallel with the edges of the wound, with no attachment to the sides of the cut. The connective tissue surrounding the trabeculæ contains numerous multinuclear giant cells. The medulla is of the red variety and blends with the osteoblastic tissue forming the trabeculæ. It is also found in some of the medullary spaces between the trabeculæ. Covering the trabeculæ, usually in a single layer, are very large polygonal cells with vesicular nuclei. These blend with the connective tissue filling in the wound.

The periosteum is thickened with the formation of trabeculæ in its deeper layer. These trabeculæ decrease as the sawcut is approached and disappear at its edge. There is possibly some absorption of the cortex, but this is questionable. Its spaces are not lined with osteoblasts. There is no absorption of the periosteal trabeculæ or of the superficial surface of the cortex.

Experiment 34. Operation performed September 7, 1905. Animal killed September 19, 1905. Fracture of twelve days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin at the site of the operation, there was no external callus to be seen, but the sawcut appeared to be filled in with a reddish-white, gelatinous material.

On longitudinal section of the bone, considerable thickening of the periosteum was found, but it was soft in consistence. The sawcut was half-filled with a firm, dull-white material, probably cartilage, which extended over one-half the distance across the medullary cavity for 15 mm. in length.

Microscopic Description. The sawcut is found to have entered the medullary cavity about 6 mm. below the entrance of the nutrient artery. The thickening felt in the periosteum is mostly of the fibrous layer, although above the sawcut, beginning at a little distance from the wound, there is a thin layer of periosteal osteoid bone formation which gradually becomes thicker and then thinner again. It has no connection with the wound.

There is a large amount of osteoid trabeculæ in the medullary cavity, the material that had appeared like cartilage being osteoid in character. This osteoid tissue extends across the lumen until it encounters the nutrient artery, which courses across the medullary cavity in a diagonal direction from above downward. This results in the formation of more osteoid tissue above than below the sawcut. The artery acts as a barrier to the formation of the osteoid tissue, as practically none is to be found at the other side of the vessel except at the point of entrance of the artery. It is difficult to state whether the osteoid tissue originates from the endosteal membrane, for that does not show exceptional thickening. The marrow itself appears to have more relation to it, there being a hyperplastic condition of the connective tissue, in addition to the fact that, on the outskirts of the callus, it is of a red, cellular variety.

The cortex exhibits some widening of the Haversian spaces, but only a very small amount of lacunar absorption with but few osteoclasts. Some of the dilated spaces may be due to its proximity to the normal cancellous tissue. They are not lined with osteoblasts. The sawcut is filled, in its lower or internal three-quarters, with osteoid trabeculæ originating from the medulla. Its upper portion is filled with blood debris, leukocytes, and phagocytes (large multinuclear cells which do not take the usual brown protoplasmic stain of osteoclasts, but more the color of blood cells). Sections where the cut has not entered the medulla show no trabeculæ. The trabeculæ in the wound go in a vertical direction, that is, parallel with the axis of the cut. The cortex edges and periosteal surface, in places, have undergone lacunar absorption by osteoclasts. All the osteoblasts are very large. There is no absorption of the periosteal trabeculæ. There are some very large multinuclear cells in the marrow.

Experiment 35. Operation performed August 30, 1905. Animal killed September 14, 1905. Fracture of fifteen days' duration.

Macroscopic Description. The wound had healed by first intention, but there was not a great amount of external callus present. On removing the skin there was some slight evidence of an old blood clot, and a small amount of callus was present at the sides of the sawcut.

Longitudinal section revealed practically no external callus, but the sawcut was filled with young bone, which occupied the medullary cavity for a space of about 1 cm. in the region of the bone wound. Next to this was an area of cartilaginous callus and then normal medulla.

Microscopic Description. There is no osteoid periosteal proliferation whatever in the celloidin sections, but the ground specimen shows, on one side of the sawcut, a moderate amount of osteoid trabeculæ. In the medullary cavity osteoid trabeculæ extend from the region of the wound almost completely across the medullary cavity and up and down from the sawcut for some distance. The endosteum is thickened and there is new bone formation lining the inside of the cortex on both sides of the canal. The layer of osteoblasts is thin. The principal and characteristic portion of the specimen is the changes that are to be noticed in the medulla. On the outskirts of the new bone growth the medulla is more cellular than normal yellow marrow. Then there appears a proliferation of connective tissue between the fat cells. This becomes gradually thicker and denser and arranges itself in the form of a more or less wide-meshed network (Fig. 13), in the same manner as the osteoid trabeculæ; that is, there is not a new cellular growth around every fat cell, but merely around groups of cells. This fact is brought out by both ground and celloidin sections. In a great measure the osteoblasts are not definitely arranged, but the bone formation is apparently by transformation of connective tissue (?). There is no absorption and no osteoclasts are apparent. Young, thin-walled bloodvessels are growing into the spaces between the trabeculæ. The oldest trabeculæ are in the region of the wound and what, on gross examination, appeared to be cartilage is young cellular trabeculæ in which there has been but little calcium salts deposited. This is demonstrated by the ground specimen.

The sawcut is, for the most part, filled with osteoid trabeculæ. These arise from the medullary cavity, as shown by their vertical arrangement and by the fact that, in the portions of the specimen where the wound had not entered the medulla, there are few, if any, osteoid trabeculæ, the wound being filled by connective tissue. The Haversian spaces in the cortex are possibly slightly dilated, but there are no proliferative changes in them.

Experiment 36. Operation performed August 30, 1905. Animal killed September 15, 1905. Fracture of sixteen days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin a small amount of callus was found at the site of operation. On longitudinal section through the bone, the sawcut was found opposite to, and 10 mm. farther up than, the entrance of the nutrient artery into the medullary cavity. The main branch artery to the upper portion of the tibia was pushed aside by the new bone formed in the medullary cavity. The sawcut was filled flush with the surface by new bone, which appeared to be cancellous in structure. There was a thickening of the periosteum which did not appear to be very firm and which did not contain new bone. On the endosteal side of the cut, the almost transparent, brownish-white, new bone formation almost occluded the lumen of the medullary cavity and extended on both sides of the cut 4 mm. Outside of this there was a very thin layer of whitish material, then red marrow with yellow marrow nearer the ends.

Microscopic Description. Only one of the celloidin sections contains a slight periosteal new bone formation. The remainder show some lacunar absorption on the surface of the cortex with, possibly, some proliferation of the osteogenetic layer of the periosteum. In the ground specimen, there is a moderate amount of periosteal new bone formation which diminishes as it approaches the sawcut and terminates at it. The sawcut is made in the portion of the bone where it is beginning to become cancellous, so that the Haversian spaces of the cortex are large. However, there is lacunar absorption by osteoclasts and in some of the spaces there are layers or sheets of large polygonal cells like osteoblasts. In some there are red marrow cells. Whether or not these spaces are the result of the fracture it is impossible to say.

The medulla is almost completely filled with osteoid trabeculæ for some distance above and below the sawcut, but not beyond the artery, which is either shoved to one side or has limited the growth of the trabeculæ. The trabeculæ are thin and covered by osteoblasts and the medullary spaces between contain young bloodvessels. In the medulla there is a formation of the connective-tissue trabeculæ that precede osteoid trabeculæ. The whitish area seen on gross examination is not cartilage, but probably connective tissue or osteoid tissue before calcareous deposition has occurred. The sawcut is filled with trabeculæ, the general trend of which is parallel to the sides of the cut. There is a great amount of lacunar absorption of the edges of the wound.

Experiment 37. Operation performed September 5, 1905. Animal killed September 22, 1905. Fracture of seventeen days' duration.

Macroscopic Description. The wound was granulating at the upper end, but there was no suppuration present. On removing the skin the granulating area was found to be bound down to the bone and was cut away. At the site of the operation, which was high up in the cancellous part of the bone, there was evidence of a small amount of callus and no evidence of the sawcut. On longitudinal section, the sawcut was found to have been made opposite to and 5 mm. higher up on the bone than the entrance of the nutrient artery into the medullary cavity. The sawcut was almost completely filled by a new bone formation which greatly resembled the old bone. There was a new formation on the endosteal side, extending not quite 1 mm. into the lumen of the cavity and extending lengthwise for about 6 mm.; 5 mm. above the sawcut, and on the outside of the bone, is new periosteal bone formation 10 mm. long and 1.5 mm. thick. Below the sawcut there was also very slight bone formation, making a hollow at the site of the operation. The periosteum was thickened all over. The marrow was red in the upper third and yellow below. It was extremely difficult to determine which was new and which old bone. The old bone was 1.5 mm. thick.

Microscopic Description. The sawcut is far up on the cancellous tissue, where the cortical covering is thin and riddled with large medullary spaces. These are filled, especially those near the medullary cavity, with eosinophilic medullary cells, bloodvessels, and loose connective tissue almost myxomatous in nature. The periosteum, above and below the sawcut, shows a rather liberal amount of osteoid trabeculæ, the axes of which are very oblique, so that they are almost parallel with the line of the bone. The spaces between them communicate in part with the Haversian canals of the cortex, as shown by the ground specimen (Fig. 14). It is probably in this manner that the bloodvessels enter the newly formed medullary spaces. Compared with that in other similar experiments, the endosteal new bone growth is small in amount, extending but a short distance into the lumen of the canal. It extends farther up and down on the inner surface of the cortex. The marrow is red, being very cellular against the new trabeculæ. There does not appear to be much connective-tissue overgrowth.

The cortex is riddled with large spaces, in some of which the continuation of the endosteum has proliferated, forming a single layer of large polygonal cells. Others contain osteoid trabeculæ, just as in the medullary cavity proper. There are some very large osteoclasts present, but there does not appear to be a great amount of lacunar absorption. The sawcut is filled with osteoid trabeculæ which appear to be derived from the widened Haversian spaces as well as from the endosteum itself. The trabeculæ are more in the form of a network, with wider spaces between them in those cases in which they are derived only from the endosteum, and they are vertically and closely arranged. It appears as if there is less new formation in the medullary canal because of the natural porosity of the region and because the physical nature of the bone does not require such a thick callus, and also owing to the fact that the cortex itself is thickened by new trabeculæ.

Experiment 38. Operation performed September 10, 1905. Animal killed September 29, 1905. Fracture of nineteen days' duration.

Macroscopic Description. The wound had united by first intention. On removing the skin at the site of operation, a small amount of callus, not very firm in consistency, was found. Apparently there was no new bone formation.

Longitudinal section through the bone showed that the sawcut had been made opposite to, and 3 mm. above, the entrance of the nutrient artery into the medullary cavity. The sawcut was filled with firm, white bone, whiter than the remainder of the leg. On the endosteal side there was a mass of callus or newly formed bone 7 mm. in length and extending over one-half the lumen of the medullary cavity, somewhat more above than below the site of the operation. On the periosteal side there was some thickening of the tissue, but no new bone.

Microscopic Description. Owing to some improper position during cutting, one end of the specimen above the sawcut consists of nothing but solid cortical bone, with no medullary cavity. The sawcut had penetrated into the medullary canal and there was a large amount of new bone formation in the medullary cavity around it, extending up into the cut flush with the surface of the bone. The bone cells in the new trabeculæ are much more abundant and have the polygonal shape of osteoblasts, differing from the spindle shape noticed in adult bone. The older of the newly formed trabeculæ contain cells more like old cortical bone cells than the younger trabeculæ, in which the cells are not compressed. The trabeculæ appear more fibrillated than usual.

The periosteum contains some osteoid bone formation, and at the edge of the wound there is a small connecting ridge between the periosteal and endosteal bone which becomes continuous. At the level of the surface of the bone the trabeculæ in the sawcut show their method of formation, having cells only partially embedded in homogeneous osteoid material (Fig. 15).

In the cortex are large Haversian spaces with little proliferation of the tissue contained therein. Some of them are due to lacunar absorption by abundant osteoclasts, especially near the periosteal surface, and some appear natural, as shown by the disposition of the bone cells around them. The marrow is not well stained.

Experiment 39. Operation performed August 29, 1905. Animal killed September 20, 1905. Fracture of twenty-two days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin no firm callus was found, but there appeared to be some thickening of the tissues in the neighborhood of the fracture.

On longitudinal section, the sawcut was found entirely filled in with a white, somewhat dense bone. On the periosteal side of the bone there was a new formation of quite compact bone above and below the sawcut, not quite 2 mm. in thickness at its thickest part. It extended upward for almost 10 mm. and downward for 5 mm., with its thickest portion about 3 mm. above the sawcut, which was filled almost flush with the level of the old bone. On the endosteal side of the cut the new bone was more cancellous and of a reddish-white color. It extended into the lumen of the medullary cavity for 2 mm. and was 10 mm. in length. The innermost shell of bone was more compact than the portion between it and the sawcut. The marrow was deep red in color excepting a very small amount at the lowermost portion.

Microscopic Description. The sawcut was made at about the junction of the cortical and cancellous bone. The periosteum shows a moderately thick layer of osteoid bone formation which extends to the sawcut and unites with that in the cut, both projecting above the surface of the old cortical bone. The periosteal new bone is very dense and the medullary or Haversian spaces between the trabeculæ are very long and narrow, running almost parallel to the old cortex. They intercommunicate with one another and with the cortex of the old bone. In the ground and cut specimen there appears to be an especially large Haversian space at the point of union of the periosteal and cortical bone, giving branches of communication to both. It shows practically no absorption.

On the medullary side of the cut there is a fair amount of osteoid bone formation which has undergone great absorption, especially on the inner margins of the mass. The phagocytic multinuclear osteoclasts are so large and so abundant as to almost form continuous layers (Fig. 16). The effects of the absorption can be seen in the form of lacunæ and large medullary spaces filled with red marrow. The surrounding marrow is red and vascular, with many free red blood corpuscles. The medullary spaces in the new medullary bone communicate freely with the old cortical Haversian spaces.

There is very great absorption of the cortical bone, the Haversian spaces being much dilated and containing abundant osteoclasts and some formative cells. Much of the tissue contained therein is almost mucoid in nature. The absorption at the saw wound is so extensive and deep that if forms communications (Fig. 17) between the old Haversian spaces and the new intertrabecular medullary spaces of the sawcut. The general trend of the trabeculæ in the sawcut is at right angles to the bone, while those of the periosteum are parallel with its axis. At the point at which they unite, neither direction is maintained, but large, irregular spaces are formed. Osteoblasts do not appear active in any other situation, and even here there is not much new bone being formed. The periosteal new bone is denser than the old cortex.

Experiment 40. Operation performed August 17, 1905. Animal died September 18, 1905. Fracture of thirty-two days' duration.

Macroscopic Description. The wound had united by first intention. On removing the skin the site of the bone wound was found to be perfectly clean, but there was not an abundance of callus present. The sawcut was completely filled in with callus.

Longitudinal section through the bone showed the operative wound to have been almost at the junction of the cancellous and the compact portion of the bone. It was located almost opposite the entrance of the nutrient artery. The sawcut was completely filled with newly formed bone, which spread out on the other side of the medullary cavity so that, on section, the whole formation of bone had the appearance of a funnel. Next to the bone there was an area of reddish, rather firm material and then normal medulla.

Microscopic . Description. The sections confirm gross examination as to the situation of the sawcut. There is a layer of callus, not of great thickness, in the periosteum above and below the cut. There is an especially large new Haversian canal present just superficial to the old cortex. The remainder of the new bone is fairly firm. Where it unites at the sawcut with the bone filling that area, there are some signs of absorption and irregularity.

In the medullary cavity there are osteoid trabeculæ extending into the lumen of the canal. These trabeculæ are wider apart and more irregular in shape than when first formed. They show signs of great absorption, being covered, in areas, by osteoclasts. These large phagocytes appear to remove the salts and liberate the bone cells intact, for in some areas the cells are one-half surrounded, the other half free and other cells seem to have been just freed. These bone cells, when liberated of their rigid surroundings, appear as moderately large mononuclear cells with small round, deeply staining nuclei and a fair amount of a rather poorly staining protoplasm. They resemble greatly normal nucleated marrow cells. The medullary spaces between the trabeculæ are very vascular and there seems to be much free blood. After the absorption of the salts the framework of the bone appears to be a loose connective tissue very much resembling its condition before calcification. This is shown in the outlying district of the new bone, in which the arrangement of the bloodvessels demonstrate that there had probably been trabeculæ present which had been absorbed. This gave the reddish, rather firm, material described above. The marrow is of the red vascular variety.

In the cortex there is a moderate amount of absorption, but not quite as much as might be supposed. There do not appear to be quite so many communications between the old and new Haversian spaces as in the previous specimen.

Experiment 41. Operation performed September 19, 1905. Animal killed November 28, 1905. Fracture of seventy days' duration.

Macroscopic Description. The wound had healed perfectly by first intention. On removing the skin, there was found, at the site of the operation, a nodule about 6 mm. in diameter.

Longitudinal section through the bone did not strike the medullary cavity throughout its entire length, but the sawcut was completely filled with hard white bone and there appeared to be some brownish, spongy bone still present in the medullary cavity.

Microscopic Description. The sawcut is healed entirely and the reparative processes in the bone have subsided greatly, there being much less activity than is seen in the early stages of repair. On the periosteal aspect there is a rather thick, very compact layer of new bone which is continuous over the sawcut as a regular surface without a depression. The external aspect shows very marked lacunar absorption (Fig. 18), the lacunæ containing osteoclasts and being filled with young cellular connective tissue. It is impossible to give the fate of the bone corpuscles, as they are liberated from the bone. They are seen first as small round cells with a small amount of protoplasm next to the bone. A little farther off only deep-blue nuclei can be seen in the midst of connective tissue. Finally, there is no sign of them whatever. Whether they degenerate and disappear or whether they go to form new connective tissue it is impossible to state, but the former seems to be the more probable. The Haversian spaces in the periosteal bone appear more like the normal Haversian spaces, being narrow and branched. In the larger ones the osteoblasts, which are still present on the sides of the canals, have lost their closely packed, polygonal arrangement, and appear more like normal medullary cells, being round, with a fair amount of indifferently staining protoplasm and a single, small, round nucleus. They look also like liberated bone cells.

The medullary cavity contains some remaining trabeculæ which are undergoing absorption, but by no means as actively as in some of the previous experiments. Some osteoclasts are present, but not in great abundance. Red cellular marrow fills in all the medullary spaces.

The sawcut is interesting and instructive. It is completely filled with more or less compact bone in which the trend of the medullary or Haversian spaces has changed from at right angles to the long axis of the bone to a direction parallel with it. Differences in staining demonstrate how the old vertical spaces had been filled in and then new transverse spaces made which were larger at first than now. There has been a new deposit of bone in both the widened canals of the old cortex and the new bone, so that an effectual union has been formed between the old and new bone. As a result of this the new bone does not separate from the sides of the cut as in previous specimens. Often, in one area, bone of three stages of development can be seen, viz., the old cortex, old new bone, and recent new bone formation (Fig. 19).

The corpuscles of this later new bone are more spindle shaped and the bone appears denser and more like the normal cortex than is the case with the older osteoid bone. Osteoblasts are not present in layers, but there appear to be more marrow cells, as described under the periosteum. All the cells look smaller than in the early active stage of proliferation.

SERIES 4.

This series, performed upon dogs, had for its object the determination of the activity of the cortex and medulla when deprived of the coöperation and assistance of the periosteum. Each operation consisted in making an incision over the tibia to the bone, reflecting the periosteum for about a half-inch on each side of the proposed cut, removing it, and then making a sawcut in the bone deep enough to enter the medullary cavity. The external wound was sutured and the leg placed in a splint and wrapped in a sodium-silicate dressing.

Experiment 42. Operation performed September 19, 1905. Animal killed September 24, 1905. Fracture of five days' duration.

Macroscopic Description. The wound had healed by first intention except at the upper angle, where a stitch had pulled out. On removing the skin, no callus was found, but the sawcut was filled with a soft, reddish material. The bone surface from which the periosteum had been denuded was granular-looking, with little crypts in it as if in a state of osteoporosis.

Longitudinal section through the bone demonstrated that the sawcut had been made opposite to, and about 2 mm. below, the entrance of the nutrient artery into the medullary cavity. The sawcut was only partially filled with a reddish-white, fairly firm material. On the endosteal side, for about 8 mm. in length and almost filling the lumen of the medullary cavity, was a rather firm, reddish-white mass, probably young callus, surrounded by a zone of reddish marrow. The periosteum was absent.

Microscopic Description. The Haversian spaces throughout the bone are very large, the cortex being comparatively thick and porous for its size. The periosteum had been effectually removed on the side of the cut, but on the opposite side of the bone and beyond the limits of its removal it gave evidences of some activity, the smooth surface of the cortex exhibiting lacunar absorption and the deeper layers of the periosteum showing some hyperplasia.

On the superficial surface of the sawcut are fibrin and red blood corpuscles Deeper down are loose connective-tissue cells with what appear to be marrow cells; and deeper still are some young osteoid trabeculæ with large osteoblasts covering their surfaces and containing large polygonal cells. These young trabeculæ appear to spring from the sides of a very large Haversian space, which communicates directly with the medullary canal and is lined with endosteum. The medullary canal contains some young trabeculæ springing from its sides in the immediate neighborhood of the sawcut. All these trabeculæ are attached to the sides of the bone and do not connect with or bridge over to one another. They are all covered with large osteoblasts, which are directly continuous with the single layer of polygonal cells lining the sides of the medullary canal for some distance from the sawcut. This is the hypertrophied endosteum.

The marrow in the region of the new tissue is very red and cellular, and in the region of the trabeculæ shows an increase of loose connective tissue. It contains numerous polynuclear cells (osteoclasts). The cortex contains large dilated Haversian spaces filled with bloodvessels, marrow cells, and loose connective-tissue cells. It is thick and has undergone some absorpton by osteoclasts. It is evidently still in the process of development, as the animal was only a pup. This absorption is general and does not appear to have any connection with the fracture.

Experiment 43. Operation performed September 15, 1905. Animal killed September 22, 1905. Fracture of seven days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin no external callus was visible. The periosteum had apparently regenerated. The sawcut was plainly visible and was filled with a reddish, soft material.

On longitudinal section through the bone, very little change was found in the medulla, as the sawcut appeared to have scarcely entered the medullary cavity. The marrow was yellow and the compact bone was very thin. There was no periosteum present.

Microscopic Description. The sawcut has barely entered the medullary cavity, pushing before it for a short distance into the canal the last thin piece of bone. However, it has traversed a couple of large Haversian spaces, which are filled with red medulla, as the sawcut has been made up near the cancellous portion of the bone. The result is the same as if it had wounded the medulla to a greater extent.

The periosteum is absent and there is no activity or absorption of the superficial portions of the bone. The medullary canal shows red marrow on the sides of the cavity, especially thick in the region of the sawcut. There is also a very thin deposit of new bone on the inner aspect of the cortex, with some proliferation of the endosteum.

The large Haversian space immediately adjoining the medullary canal shows thickening of the endosteum, with the formation of new trabeculæ, more or less fibrous in nature and covered with osteoblasts. The space contains red marrow cells. The sawcut is filled with loose connective-tissue cells, some marrow cells, and trabeculæ.

Experiment 44. Operation performed September 21, 1905. Animal killed September 30, 1905. Fracture of nine days' duration.

Macroscopic Description. The wound was found to have healed by first intention. On removing the skin there did not appear to be any callus at the site of the operative wound, but the sawcut was filled flush with the surface of the bone with a somewhat soft, whitish material.

Through some oversight in sectioning the bone longitudinally, it was taken in the wrong direction, so that the entire operative area was in one-half of the bone. The section was cut in half through the sawcut and preserved for embedding in that manner. There was thick, white tissue on the outside and thickening in the medullary cavity in the neighborhood of the wound, but no new bone formation. The operative wound was half-filled with a rather firm, white material.

Microscopic Description. The sections were made through one-half of the bone opposite to where the operation was performed, so that the cut was not in the section at all. There was no periosteal proliferation to be seen. The medullary cavity contains bone formation on the inner aspect of the cortex, projecting into the lumen of the canal for a short distance, with some cellular marrow in its neighborhood. In the centre of the canal, in some of the sections, there are a few osteoid trabeculæ, demonstrating that the nodule that was present at the site of the cut had extended over half the distance across the canal. The specimen shows beautifully the filamentous connective tissue between the fat cells (Fig. 11) on the outskirts of the mass of osteoid trabeculæ, illustrating how this increases in quantity, gradually obliterating the fat cells and then becoming converted into osteoid trabeculæ by the invasion of the osteoblasts and a deposition of calcareous salts. In the ground section osteoid trabeculæ fill in the sawcut, but there is no periosteal activity and no cortical changes are to be seen.

Experiment 45. Operation performed September 8, 1905. Animal killed September 20, 1905. Fracture of twelve days' duration.

Macroscopic Description. The wound had healed by first intention, but at the upper end a stitch had broken and there was a clean, granulating area, covered with a scab. No pus was present. At the site of the operative wound no callus could be noticed, but at the ends, where the periosteum had been cut off, some thickening could be felt.

Longitudinal section through the bone revealed the fact that the sawcut had been made opposite to the entrance of the nutrient artery into the medullary cavity. The sawcut was filled flush with the surface of the old bone with yellowish-white, cancellous bone which extended into the lumen of the medullary canal 2 mm., but was not quite 4 mm. in length, extending equally above and below the sawcut. The nutrient artery gave off its large branch to the upper end of the bone directly opposite to this mass. The marrow was red excepting in the lowermost portion of the bone, where it was pink. Where the periosteum had been cut off, 9 mm. above the sawcut, there was a new formation of bone 1 mm. thick, extending upward 15 mm. There was no new bone formation from the lower portion.

Microscopic Description. The sawcut has entered the medullary cavity and has given rise to an abundance of osteoid trabeculæ. This mass extends about half-way across the lumen of the medullary canal. It is composed of a network of osteoid trabeculæ with fairly large medullary spaces between, containing a lining layer of polygonal cells (osteoblasts) and, in the centre, loose connective tissue and engorged thin-walled bloodvessels. On the outskirts of the rounded mass of callus there is, in places, a layer of loose connective tissue and then red marrow containing greatly engorged bloodvessels. There are some osteoclasts to be seen in the medullary spaces between the trabeculæ. In a few of the dilated Haversian spaces of the cortex next to the medullary cavity there is hyperplasia of the endosteum, with osteoid trabeculæ formation where they have been cut across. The cortex shows great absorption, especially near the superficial portions of the bone and in the neighborhood of the sawcut; also a great number of osteoclasts. In areas the absorption extends well into the cortex, forming new, wide Haversian spaces, which connect with the new medullary spaces between the trabeculæ in the sawcut. On the periosteal surface there is an abundance of osteoid tissue at a distance from the sawcut where the periosteum had been cut off at the time of operation. The raised periosteum is hyperplastic in its deeper layers. The general trend of the trabeculæ is away from the sawcut. The explanation of this direction may be in the fact that the periosteum proliferates and forms trabeculæ, but does not grow in length, so that, when it is raised up, it swings as on a pivot and its end is opposite a relatively higher point on the bone, giving a diagonal direction to the trabeculæ. The cortex, from which the periosteum has been removed, exhibits some lacunar absorption, but it is not very marked, being from the body of the cortex outward and not originating on the surface. On the other side of the sawcut also, just in continuity with the tissue in the cut, are some osteoid trabeculæ. These do not appear to be periosteal in origin, because the general trend of the trabeculæ is toward the sawcut and not away from it. In addition the periosteum had been removed at the time of operation. Moreover, the trabeculæ are young, which would not be the case were they from the periosteum.

The superficial surface of the cortex on the side opposite to the sawcut shows lacunar absorption by osteoclasts and some proliferation of the deeper layers of the periosteum, but no new formation.

Experiment 46. Operation performed September 5, 1905. Animal killed September 20, 1905. Fracture of fifteen days' duration.

Macroscopic Description. The wound was entirely healed. On removal of the skin no callus but some slight thickening was found at the site of operation; also some tissue of a yellowish-white color.

The sawcut was filled flush with the surface by brownish-white, cancellous bone which extended into the medullary cavity and entirely occluded the lumen, extending 7 mm.

longitudinally. Next to this, toward the lower ends of the bone, was a small area of a firm, whitish material, possibly cartilage, and then whitish marrow. The marrow of this bone does not appear to be congested. The periosteum was thickened where it had been cut off, but there were no signs of bone formation.

Microscopic Description. The periosteum, which was completely removed at operation, has not regenerated. The medullary cavity in the region of the sawcut is almost completely filled by osteoid trabeculæ, between which are large medullary spaces that are filled with loose connective tissue and numerous osteoclasts. The absorption of the trabeculæ is well under way. There is a very small amount of red marrow and some congested bloodvessels around the osteoid mass. The nutrient artery runs through the mass of osteoid tissue and demonstrates clearly that the adventitia of the bloodvessels takes no part in bone formation.

The sawcut is completely filled with osteoid trabeculæ, which do not appear to be very thick. The general direction of the trabeculæ is vertical to the line of the bone. There are some osteoclasts present, but not in such great abundance as in the medulla. The osteoid mass extends above the level of the bone and exhibits a tendency to "overflow" above and below the cut, as described in the previous specimens showing trabeculæ of endosteal origin on the periosteal aspect. There is little if any absorption of the cortex.

Experiment 47. Operation performed August 31, 1905. Animal killed September 19, 1905. Fracture of nineteen days' duration.

Macroscopic Description. The wound had healed by first intention. At the site of the bone wound nothing could be found, the bone apparently having entirely healed, leaving only a slight elevation at the site of operation.

On longitudinal section through the bone, practically no thickening was found on the periosteal side of the sawcut. The entire cut was filled flush with the surface by a brownish-white, cancellous bone which extended half-way across the lumen of the medullary canal. It was 10 mm. in length, with the greater part toward the upper end, following a branch of the nutrient artery. Next to the new bone there was a layer of a cartilaginous-looking material, and then red marrow. The principal branch of the nutrient artery going to the upper extremity of the bone runs through the callus.

Microscopic Description. Probably the periosteum had not been thoroughly removed a little to one side of the wound of the bone, as there is a small deposit of new bone at some distance from the sawcut which is distinctly localized. It is probably due to the leaving of the lower layer of the periosteum, as that membrane cannot be seen remaining in its entirety. The lack of definite shape and direction of the trabeculæ confirm this conclusion.

The medullary cavity, in the region of the sawcut, is almost completely filled with osteoid tissue, the greater part of which is above the sawcut. Apparently, it is broken up into islands by branching bloodvessels, each island being surrounded by loose connective tissue or myxomatous tissue. The trabeculæ do not approach the vessels. Osteoclasts are present in moderate numbers. The sawcut is filled with osteoid trabeculæ, taking a vertical direction, extending above the level of the cut, and, as in the previous section, "overflowing" it on one side only, the direction of the trabeculæ being continuous with those in the sawcut (Fig. 20).

The bone itself shows little absorption, there being no communications between the medullary spaces of tne osteoid tissue and the Haversian spaces. In the medulla, the whitish area noticed around the bone mass, is loose, myxomatous tissue surrounded by cellular red marrow.

Experiment 48. Operation performed August 22, 1905. Animal killed September 14, 1905. Fracture of twenty-three days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin there was found a small amount of callus at the site of the operation

Longitudinal section through the bone demonstrated that the sawcut had been made opposite to and a little higher than the entrance of the nutrient artery into the medullary cavity. The sawcut was completely filled with a rather compact-looking bone which was slightly yellower in color than the old compact bone. On the periosteal side of the bone there was some soft, white material which appeared as if it might have contained cartilage, but did not look like typical callus. On the endosteal side of the sawcut there was a new formation of cancellous bone occluding one-half of the lumen of the medullary canal and extending 3 mm. above and 13 mm. below the sawcut in the direction the nutrient artery takes in the bone. Next to the bone there was a very thin layer of white, cartilaginous-looking material, and then congested marrow.

Microscopic Description. The sawcut has entered the medullary cavity and the medullary osteoid tissue is mostly in one direction from the sawcut. The osteoid tissue is more or less divided into masses with myxomatous tissue and red marrow on the edges There are some osteoclasts present, but not in great abundance, and there is not much absorption of the internal callus. The sawcut is filled with osteoid tissue which is rather compact, but the direction of the medullary spaces is more transverse than usual. Near the periosteal aspect there is great absorption of the cortex, which blends with the new osteoid tissue, uniting the medullary spaces of the old and the new tissue (Fig. 21). Osteoclasts and osteoblasts are present in these spaces. There is osteoid bone formation in the periosteum, where it still remains attached.

Experiment 49 Operation performed July 6, 1905. Animal killed August 3, 1905 Fracture of twenty-eight days' duration.

Macroscopic Description. There were almost no signs of the operation, only a narrow, white, linear scar at the site of the operative wound. Feeling through the scar, there was at the site of the bone wound a slight depression with a small elevation on each side. After removal of the skin the bone was found to be recovered by a thin, filmy membrane, reënforced at the site of the operation by a thick, tough material. The cut was almost completely filled in, although evidence of it was still present.

On longitudinal section almost the entire medullary cavity of the bone was found to be filled by a firm callus, appearing as if it contained osteoid trabeculæ. The callus toward the lower extremity of the bone originated from that in the neighborhood of the sawcut and had grown into the medulla, for it was not attached to the endosteum at the sides, but appeared to be surrounded by dark-brown marrow. A rather large collection of marrow was found between the two portions, which connected with each other.

Microscopic Description. The ground specimen shows that the sawcut had entered the medullary cavity at one point, but not very deeply. Almost the entire cavity is filled with osteoid trabeculæ in separate batches or islands. They show some absorption, but not a very great amount. Some osteoclasts are present. Surrounding these masses is filamentous tissue containing some marrow cells. The sawcut is filled with osteoid trabeculæ which are continuous, with a considerable proliferation on the periosteal aspect, the origin of which it is impossible to determine. The trabeculæ fill in the sawcut on the sides, but not completely in the centre of the cut. The cortex shows widened spaces which communicate directly with the medullary spaces in the new trabeculæ. Osteoclasts are present in moderate numbers. Osteoblasts are abundant.

Among the osteoblasts and in the medullary spaces of the superficial portions of the callus there appears to be considerable hyaline material taking a diffuse eosin staining.

It is present in the form of round masses or strands. What it is cannot be determined, but it looks as if it may be a homogeneous hyaline secretion from the osteoblasts.

Experiment 50. Operation performed August 17, 1905. Animal killed October 5, 1905. Fracture of forty-nine days' duration.

Macroscopic Description. The wound was found to have healed by first intention. There was considerable thickening of a somewhat cartilaginous nature directly over the sawcut, but it appeared to be somewhat movable; so that its nature could not be determined until section was made

On longitudinal section through the bone, the periosteum was found to have apparently regenerated and was considerably thickened over the entire length of the specimen. The sawcut was entirely filled with whitish, hard bone, not so compact as the normal bone. The new bone extended about half-way across the medullary cavity and on each side of the sawcut for about 3 mm. Surrounding the new bone was red marrow and farther down yellow marrow.

Microscopic Description. The sections demonstrate that the gross description was in error, for there is no regrowth of the periosteum itself, but only of connective tissue There is absolutely no new periosteal bone formation, but some lacunar absorption, making the superficial cortical surface rough.

In the medullary cavity there is a moderate amount of osteoid tissue which extends about half-way across the lumen of the canal. It is quite dense in structure, the medullary spaces being quite small and the trabeculæ thick There is very little activity evident, there being no osteoblasts on the medullary trabeculæ and only a very moderate amount of osteoclasts, causing slow absorption of the endosteal callus. The medulla contains some marrow cells, but is not extremely cellular as in active states.

The sawcut is filled flush with the surface of the bone with osteoid tissue which is rather compact, but in which the general trend of the medullary spaces is becoming in the line of the bone instead of at right angles to it. There is distinct evidence of the former direction of the old trabeculæ in the streaks present in the new trabeculæ. Quite cellular, new, young bone fills in the sites of the old spaces. The new spaces are lined by osteoblasts and are directly continuous with the widened Haversian spaces of the old cortex (Fig. 32). They show a deposit of still younger new bone The cortex is rarefied.

SERIES 5.

This series, for which dogs were used, was performed with the object of determining the activity of the bone itself when stripped of its periosteum and when not accompanied by visible injury to the medulla. Each operation consisted in making an incision over the tibia to the bone, reflecting the periosteum for about 2.5 cm., and then cutting off the reflected portion. In this denuded area of the cortex, one or two shallow sawcuts, not deep enough to enter the medullary cavity, were made. The wound was then sutured and the leg dressed as described under previous operations.

Experiment 51. Operation performed September 13, 1905. Animal killed September 16, 1905. Fracture of three days' duration.

Macroscopic Description. The dressing was full of blood and the wound still open. On removing the skin there was found a very large hematoma extending from the knee to the ankle. There was no infection. Longitudinal section showed the sawcut to be filled with blood. The medulla did not appear to be affected.

Microscopic Description. The sawcuts are filled with red blood corpuscles, a small amount of fibrin, and a few leukocytes. The periosteum has been stripped away and the surface of the cortex is smooth. There is little or no alteration of the medulla excepting, possibly, a slight increase in the congestion and free blood cells at the site of the canal immediately adjoining the cuts. This may have been produced by the fact that a vessel leaving the canal runs almost directly across the bone to the region of the cuts.

There are a few dilated spaces in the cortex containing osteoclasts and a lining of osteoblasts. These are found near the cuts in the bone, but also in other portions removed from the site cf operation. The bone looks as if it had been the seat of recent activity because of an entirely distinct, rather thick layer of apparently new bone lining the medullary cavity. This formation has nothing to do with the operation performed for these experiments.

Experiment 52. Operation performed June 20, 1905. Animal killed June 25, 1905. Fracture of five days' duration.

Macroscopic Description. The wound was perfectly clean, healing progressing by first intention. There were some signs of organizing blood clot on the inner aspect of the bone. In the sawcut there was very little evidence of an effort on the part of nature to throw out callus The sawcut contained some blood clot which was not disturbed.

Microscopic Description. The sections did not enter the medullary cavity, so its description must be omitted. The sawcut appears to be filled with red blood corpuscles and a small amount of fibrin. There are no evidences of the periosteum. There is no roughening or absorption of the superficial aspect of the cortex, or of the sides of the sawcut. The cortex shows possibly slight widening of the Haversian spaces, but to no great extent. There is no lacunal absorption to be noted and no osteoclasts are present. The tissue surrounding the bloodvessels in the Haversian canals exhibits some proliferation, but not sufficient to form a layer of osteoblasts.

Experiment 53. Operation performed September 12, 1905. Animal killed September 24, 1905. Fracture of twelve days' duration.

Macroscopic Description. The wound was found to have healed at all points excepting the upper extremity, which had pulled apart and was healing by granulations. At the site of the bone wound everything was covered with connective tissue, so that nothing definite could be distinguished.

Longitudinal section through the bone showed the sawcut to have been made opposite to and 3 mm. above the point of entrance of the nutrient artery into the medullary cavity. In the section made the sawcut did not appear to have entered the medulla, but the medullary cavity in the region of the cut contained new bone or osteoid tissue 1.5 mm. thick and 10 mm. long in the direction of the nutrient artery. The medulla was red. The sawcut was filled with a somewhat reddish material, but no bone. The periosteum, 5 mm. above the sawcut, was 2 mm. thick and appeared to contain cartilage; below it did not. It appeared as if the periosteum had regrown over the sawcut.

Microscopic Description. The entire cortex is absolutely riddled with very wide Haversian spaces, which show, in some places, very large numbers of osteoclasts, one small space containing eleven of them. All these dilated spaces contain an abundance of loose myxomatous tissue in addition to bloodvessels, some marrow cells, and a layer of osteoblasts. The sawcut is absorbed to so great an extent that it can hardly be recognized as a straight cut. Spicules of bone around a depression show where it had been made. While in none of the sections has it penetrated the medullary canal, it has approached very close to it and has cut across a couple of Haversian canals, which go directly into the canal from the cut, forming narrow communications between the two.

The periosteum contains a fair amount of osteoid tissue at a distance from the wound and a thin, irregular layer up to the edge of the cut. The sawcut also contains some trabeculæ which do not stream upward as in other series, but form large, round canals lined with osteoblasts and connective tissue. Whether these trabeculæ and the thin layer on the periosteal surface arise from cells of the deeper layer of the periosteum inadvertently left remaining, or are from the

Haversian canals of the bone, it is impossible to determine.

The irritation has extended also into the medulla by referred stimulus along the communicating Haversian spaces. There is a nodule of osteoid trabeculæ in the medullary canal opposite to the sawcut. It differs from previous endosteal growths in not being irregular, but distinctly limited by a membrane resembling the deeper layer of the periosteum (endosteum) (Fig. 24). The medulla is red and cellular, but does not show web-like formation of connective tissue.

Experiment 54. Operation performed August 29, 1905. Animal killed September 22, 1905. Fracture of twenty-four days' duration.

Macroscopic Description. The wound had healed perfectly. There was no pus present. There was no callus formation, but some thickening at the ends of the periosteum where it had been cut off. The sawcut was still plainly visible and not completely filled in except by a reddish material.

Longitudinal section revealed the fact that the sawcut was very shallow and had been made 5 mm. lower down than the entrance of the nutrient artery into the medullary canal. The medulla was not apparently affected by it. The cut was filled with white and brown material, not very firm in consistence. Where the periosteum had been cut off 10 mm. below the sawcut, there was a very thin formation of new white bone not 1 mm. in thickness and extending for about 10 mm. down the bone. There was a suggestion of the same thing at the upper end. The bone-marrow was red in the upper three-fifths and yellow below.

Microscopic Description. The sawcut is rather shallow. The periosteum, where it had been left remaining, shows the formation of a layer of osteoid trabeculæ of fair size. There are no signs of periosteum over the portion where it had been removed at the operation, and there is no superficial lacunar absorption. The sawcut is filled with young fibroblasts and bloodvessels, *i. e.*, granulation tissue with some slight traces of the original blood clot remaining.

There is very marked absorption of the cortex, with very large dilated Haversian spaces containing osteoclasts, a lining membrane of osteoblasts, loose connective tissue of myxomatous nature, and bloodvessels. The medulla is of the red, cellular variety. On the medullary aspect of the cortex, at the site of the saw wound, is a small, flat elevation or nodule of newly formed osteoid trabeculæ (Fig. 23), lined with polygonal osteoblasts, around which is a thin layer of connective tissue. Outside of this is red marrow. There are no osteoid trabeculæ in the marrow detached from the endosteum, demonstrating clearly that the endosteum has been stimulated to growth by the injury to the bone above.

Experiment 55. Operation performed July 3, 1905. Animal killed August 2, 1905. Fracture of thirty days' duration.

Macroscopic Description. The wound had healed by first intention. There was no callus visible, but the periosteum (?) had again apparently covered the bone and was continuous over the wound in the bone as a thin, film-like membrane.

Microscopic Description. The sawcut has not penetrated into the medullary cavity. There is no periosteal new bone formation or absorption, the surface being smooth. There is a fibrous tissue covering the bone, but whether it is periosteum or not it is impossible to state. The sawcut is filled with cellular, fibrous tissue like granulation tissue. Some of the deeper cells are very large, with oval, vesicular nuclei. One side of the sawcut shows marked lacunar absorption apparently by osteoclasts. A small portion of the wound looks as if there is a beginning deposition of new bone lined with a layer of osteoblasts. Their origin is unknown, but it is doubtful if it is periosteal, because the periosteum exhibits no signs of activity elsewhere. There is a tendency to the formation of cancellous tissue on the medullary aspect of the cortex, there being large spaces filled with cellular marrow.

The medulla is red and cellular only in the neighborhood of the sawcut. There is no apparent new bone formation. There is only a very moderate amount of absorption of the cortex.

Experiment 56. Operation performed August 18, 1905 Animal killed September 22, 1905. Fracture of thirty-five days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin there was found practically no effort at repair, no callus being present. The sawcut was perfectly visible.

Longitudinal section through the bone demonstrated that the sawcut had been made opposite to and 10 mm. farther up on the bone than the entrance of the nutrient artery into the medullary cavity. The medullary cavity was not at all changed, but the marrow was red. The sawcut did not enter the medulla. The periosteum had apparently regrown over the portion where it had been denuded and filled in the sawcut, but there was no attempt at callus formation.

Microscopic Description. The sawcut is shallow, not penetrating far into the cortex. There is a formation of osteoid trabeculæ in the periosteum, where it still remains attached at some distance from the sawcut. There are no trabeculæ near the sawcut. The cut is filled in, apparently with granulation tissue. There is absorption of the sides of the cut.

The cortex shows great absorption with widened Haversian spaces lined by osteoclasts. The marrow is red and cellular. On the medullary side of the cortex, in the region of the sawcut, there is a small deposit of newly formed osteoid trabeculæ. The marrow exhibits no connective-tissue hyperplasia in that region.

Experiment 57. Operation performed June 20, 1905. Animal killed August 4, 1905. Fracture of forty-five days' duration.

Macroscopic Description. There was superficial sloughing of the tissues on the outer aspect of the thigh. The wound itself was unaffected. At the site of fracture, on removal of the skin, there was found no evidence of the sawcut, but the area was covered with a cartilaginous material which was probably callus from regenerated periosteum.

On section through the bone there was no evidence of the sawcut excepting a narrow, dark spot on the upper end, in the cancellous portion.

Microscopic Description. The sawcut has not entered the medullary cavity. The periosteum contains some new bone formation at a distance from the wound, but none near it. The sawcut is filled by osteoid trabeculæ having a transverse direction of the spaces between them. There are evidences that the original direction was in the line of the cut. The cortex has undergone great absorption, especially between the bottom of the sawcut and the medulla (Fig. 25). The spaces are very wide and form communications between the cut and the medullary side of the bone. The medullary cavity contains a considerable amount of osteoid tissue in the region of the sawcut. As shown by the ground specimen, this tissue has large medullary spaces. The medulla is red.

Experiment 58. Operation performed August 17, 1905. Animal killed October 26, 1905. Fracture of seventy days' duration.

Macroscopic Description. The wound was found to have healed by first intention. The bone was completely covered by tissue, which was probably periosteum. At one point, doubtless the site of operation, there was a moderate elevation quite hard in consistency, which may have been callus or bone.

Section in a longitudinal direction showed practically no signs of the operation on the bone, which was performed opposite to the entrance of the nutrient artery into the medullary cavity. The sawcut was entirely filled with hard tissue. There was no apparent new bone formation on the endosteal side, but there was a layer of red marrow 1.5 mm. thick on the side of the sawcut, beyond which was yellow marrow.

Microscopic Description. The sawcut is completely filled, flush with the surface of the bone, by new compact bone which has fewer medullary spaces than the surrounding cortex (Fig. 26). There was probably an excess, which has been removed, as the periosteal aspect of the new bone shows osteoclastic lacunar absorption (Fig. 27), as does the surface of the cortex. The medullary spaces of the new bone have a tendency to communicate with those of the cortex. The cortex has undergone considerable absorption, the dilated spaces often containing fat and medullary cells, but no osteoblasts as distinct cell layers. Osteoclasts are not found in the cortex. The cortex, under the periosteum where it still remains, distant from the cut, exhibits considerable osteoclastic absorption. There is a new bone formation of moderate size in the medullary cavity against the inner side of the cortex in the region of the sawcut. It has suffered considerable osteoclastic absorption. The medulla is cellular, but not to the extent of obliterating the fat cells.

SERIES 6.

This series, upon dogs, was performed with the object of determining the activity of the periosteum, when raised from the bone and accompanied by injury to the bone not deep enough to penetrate the medullary cavity. Each operation consisted in making an incision over the tibia to the bone, and then, in most cases, raising the periosteum from the bone by making an incision in the line of the bone and passing a periosteal elevator under the membrane. After raising the periosteum, a saw was passed under it and a couple of very shallow sawcuts made in the bone, and the periosteum again allowed to fall back into place. In a couple of instances the periosteum was cut across, and after making the injury to the bone was sutured in place by catgut. Before closing the wound the periosteum was tapped sharply several times with some blunt instrument to cause more injury. The wound was then sutured with silk and covered with collodion.

Experiment 59. Operation performed February 19, 1906. Animal killed February 21, 1906. Fracture of two days' duration.

Macroscopic Description. The wound was found to be clean, with the sutures and collodion still in place. On removing the skin at the site of the operation, the periosteum was seen to be raised up and thickened and was of a reddish appearance, as if it consisted of organized blood clot. It was quite firm in consistency and extended beyond the confines of the bone.

Longitudinal section through the bone confirmed the findings before ection. The medulla was pink in color. There were no changes in the medullary cavity.

Microscopic Description. There is a large amount of blood clot with fibrin and an abundance of polymorphonuclear leukocytes. Under the raised periosteum are several areas of hyaline, fibrillar material, showing a small amount of leukocytic infiltration. The bone is not altered; the medulla is slightly increased in cellular content, but is not red, cellular marrow. Some of the new cells are fairly large.

Experiment 60. Operation performed February 21, 1906. Animal killed February 24, 1906. Fracture of three days' duration.

Macroscopic Description. The wound was found to be perfectly clean, but not completely united, as the edges could be pulled apart with the escape of a small amount of serum. On removing the skin, the periosteum, at the site of the operation, appeared to be thickened, red and edematous, and did not appear to be attached to the underlying bone. The sawcuts could be distinctly felt.

Longitudinal section through the bone showed the periosteum to be thickened, but there was no alteration in the bone. The medulla was red.

Microscopic Description. The sawcuts are very shallow and are covered over by periosteum, which remains as a fibrous strip across the top. The cuts themselves are filled with blood clot (red blood corpuscles, fibrin, and leukocytes). The connective tissue outside the periosteum exhibits an abundant formation of young fibroblasts, which resemble closely portions of the deeper layer of the periosteum.

The periosteum itself, where it has been raised, remains as a distinct fibrous layer with no proliferation. Where it has remained attached to the bone, there is proliferation of both the superficial fibrous layer and of the deeper osteogenetic layer. The appearance of this deeper layer is rather odd. The cells gradually become larger as one goes deeper, until they appear to resemble large marrow cells, being loose in their disposition and not made polygonal by mutual compression. Several Haversian spaces, lined with similar cells, communicate with this layer of the periosteum. At one place there is just the beginning of the formation of bone. The superficial layers of the old bone cells appear somewhat larger and more vesicular-looking than the remainder. Some seem to be breaking loose and entering the periosteal tissue.

There is widening of the Haversian spaces of the cortex. These spaces contain abundant cellular tissue resembling marrow cells, which are showing a tendency to form layers around the sides. The nuclei of the bone cells around these spaces appear larger and more vesicular than those farther removed. Osteoclasts are present.

The medulla is very cellular. It contains cells of varying sizes, some small round cells with deeply staining, single nuclei (occasionally eccentrically placed); others, cells of the same nature, but larger and with more protoplasm; still others, with larger vesicular nuclei, and, finally, very large cells with a great abundance of protoplasm and four to ten rather large and vesicular nuclei. These cells are probably forming osteoclasts, derived from the last of the above-named cells. Haversian spaces connecting with the medullary cavity show the medullary cells streaming into them.

Experiment 61 Operation performed October 20, 1905. Animal killed October 24, 1905. Fracture of four days' duration.

Macroscopic Description. The wound was found to have healed by first intention. At the site of the operation there was a great amount of blood.

On section through the bone a blood clot was found under the periosteum at the site of the operation. The medulla was red but there was no evidence of proliferation.

Microscopic Description. The periosteum cannot be made out definitely as a distinct layer, but there are numerous thick bands of fibrous tissue to be seen. There is no proliferation present, but sections of the catgut probably identify these bands as periosteum. There is no roughening of the periosteal aspect of the cortex excepting where the periosteum is still attached. There is no new trabeculæ formation anywhere excepting on the side opposite to where the operation was performed. Here there is slight periosteal proliferation. The sawcut and all the tissues on the periosteal aspect are filled with blood.

The cortex of the entire bone shows great rarefaction, but few osteoclasts. These spaces are filled with an abundance of round or oval cells resembling marrow or liberated bone cells, also dilated bloodvessels. The medulla is red, containing many free red blood corpuscles. Fat is also present. There are, in addition, numerous round or oval cells, varying in size, with small or moderate-sized, round, well-staining nuclei. Especially toward the ends of the bone, some of these cells exhibit a slight increase of the protoplasm, but, what is more noteworthy, is an apparent division of the nuclei into two or four parts They appear as if they may be a forerunner of the osteoclasts a few of which are to be found in the medulla.

Experiment 62. Operation performed February 16, 1906. Animal killed February 24, 1906. Fracture of eight days' duration.

Macroscopic Description. The wound had healed by first intention. On removing the skin the periosteum was found to have been raised up and thickened and was reddish white in color. A small amount of pink serum escaped when the skin was removed. Some irregularities could be felt on the bone surface

Longitudinal section through the bone showed great thickening of the periosteum, with no apparent callus or new bone formation. The marrow was reddish in color. The bone seemed rather porous.

Microscopic Description. The periosteum was present as a distinct continuous layer, fibrous tissue above and markedly proliferated cells below. The osteogenetic layer, for the entire length of the bone exhibits considerable cellular proliferation, which dips down into the sawcuts. One cut contains blood and fibrin, being displaced by an ingrowth of this tissue There are osteoid trabeculæ in the periosteal tissue up near one of the sawcuts, and a beginning formation of them can be found in the bottom of it. There is great absorption of the entire cortex by osteoclasts. Cells are arranging themselves in layers along the sides of the widened canals. The medulla is red and contains many very large multinuclear cells (osteoclasts). There is a formation of osteoid trabeculæ on the medullary aspect of the cortex on the side of and in the region of the sawcut.

Experiment 63. Operation performed February 23, 1906. Animal killed March 5, 1906. Fracture of ten days' duration.

Macroscopic Description. The wound had not closed, the stitches having been torn out. It was healing by granulation. There was no pus visible and there was no apparent change in the bone even after removing the skin.

On longitudinal section, slight thickening of the periosteum was found at the site of the operation, but there was no apparent callus to be seen. The medulla was not affected.

Microscopic Description. Owing to the fact that it was practically a compound fracture, there is such an abundance of young fibrous tissue that it is difficult to distinguish periosteal tissue from the remainder. However, there is tissue denser in some places than others, forming a layer which is probably the fibrous portion of the periosteum. Beneath it there is an abundance of young fibrous tissue or osteogenetic tissue, its exact nature not being determinable owing to the fact that the cells are more closely packed and not quite so large as is usual with osteoblasts. In addition, there are present more bloodvessels than is usual.

In areas there is lacunar absorption of the superficial portions of the cortex. There are some areas that resemble typical portions of the proliferated osteogenetic layer of the periosteum. At the base of one of the cuts and in a couple of additional areas, there is just the beginning formation of trabeculæ. The cortex proper contains an abundance of osteoclasts and a few large spaces caused by lacunar absorption. Some of these connect with the periosteum. They are lined by osteoclasts. The medulla is red and congested, but many fat cells remain. It shows a moderate amount of osteoclasts. On the endosteal surface of the cortex is a layer of cells resembling osteoblasts, but no endosteal trabeculæ.

Experiment 64. Operation performed March 2, 1906. Animal killed March 16, 1906. Fracture of fourteen days' duration.

Macroscopic Description. The wound had united perfectly excepting over the upper portion, where there was a granulating area, but no pus present. This area did not appear to communicate with the bone. On removal of the skin there was found a slight elevation at the site of the operation on the bone, but no callus was apparent.

On longitudinal section of the bone, slight thickening of the tissues over the site of the operation on the bone was found, but no callus. The bone was very small and the medullary cavity very narrow. The medulla was quite red throughout its entire extent.

Microscopic Description. The periosteum is present as a distinct layer and the sawcuts are very shallow. The deeper layer of the periosteum is proliferated and fills in the sawcuts with osteoblasts. There is a new osteoid bone formation from the periosteum extending up to the edge of the sawcuts. Its trabeculæ are covered with osteoblasts. In areas where there are no trabeculæ there is lacunar absorption of the surface of the cortex. These areas are covered by a layer of large cells, with large, oval, vesicular nuclei. There are a few osteoclasts to be seen. Absorption is evident in the sawcuts. There is practically no absorption of the cortex itself and its spaces do not appear to be lined by osteoclasts.

The medulla shows only a slight increase in its cellular content. It is most marked near the medullary border of the cortex, where there are some large cells with single, large, round, vesicular nuclei. No osteoblasts can be seen in the medulla.

Experiment 65. Operation performed February 2, 1906. Animal killed February 21, 1906. Fracture of nineteen days' duration.

Macroscopic Description. The wound had healed perfectly. At the site of the operation there was a marked nodulation, fairly firm in consistency, and not entirely adherent to the scar. Through some error, on removing the skin this nodule was cut across. It was fairly firm, but looked more like granulation tissue than cartilage. The bone was still bare, the periosteum not being adherent to it. The sawcuts were plainly visible. The entire portion of the periosteum, that had been raised, was considerably thickened.

Longitudinal section showed the periosteum to be thickened and not adherent to the injured bone. Toward the lower end of the bone, where the periosteum was still attached, there was a small area of periosteal new bone formation about 1 cm. in length and 1 mm. thick. There was a thin layer of new cancellous bone in the medullary cavity opposite to the site of the operation.

Microscopic Description. The periosteum is raised up and not attached to the bone except at the ends where it had been left at the operation. At both of these situations there is a large formation of osteoid tissue. Its trabeculæ are covered with osteoblasts and also numerous osteoclasts. Where the periosteum had been raised up, it is greatly thickened and contains abundant fibroblasts, that is, large spindle cells with oval, vesicular nuclei. These are not arranged in a single layer, all going in the same direction, but appear to course in all directions. Some of the cells are especially large and, in one area, there appears to be an attempt at trabeculæ formation which is not, however, impregnated with calcareous salts, but merely hyaline in appearance. There is a moderate leukocytic infiltration present.

Some portions of the superficial aspect of the cortex have undergone absorption. The sawcuts show no absorption. There is moderate absorption of the cortex proper, its spaces being lined with osteoblasts.

In the medulla there is a rather large growth of osteoid trabeculæ, covered with large osteoblasts. No osteoclasts can be noted among the trabeculæ or in the marrow itself. The marrow is more cellular than normal, but not very red. There is connective-tissue hyperplasia of the marrow in the region of the osteoid tissue.

Experiment 66. Operation performed January 26, 1906. Animal killed February 24, 1906. Fracture of twenty-nine days' duration.

Macroscopic Description. The wound had healed by first intention. The scar was not adherent to the underlying tissue. Examination revealed an apparent thickening on the bone at the site of the operation and a small nodule, somewhat movable, on the anterior aspect of the bone, probably at the site of a catgut suture. On removal of the skin the inequalities of the surface of the bone could no longer be detected. The thickening was not appreciable to the naked eye. The small nodule mentioned was soft and yellowish in color.

Longitudinal section through the bone showed thickening of the periosteum at the site of the operation. The cuts were no longer visible in the bone, but there was present what was possibly a very thin layer of cartilage. The bone was a little thicker at the site of the operation, but it was impossible to say if there was new bone present. The medulla was reddish in color.

Microscopic Description. The periosteum is apparent as a distinct layer, but mostly fibrous in nature. There is not much activity present, it having already ceased. There is an abundance of periosteal osteoid trabeculæ above and below the site of operation, and less at the operative wound. The principal activity to be seen now is great absorption on the surface of the newly formed trabeculæ and the formation of wide, longitudinal Haversian canals in its deeper layers. Large osteoclasts are present in great abundance, and also a thin layer of apparently inactive osteoblasts. The spaces in the periosteal trabeculæ, as well as in the cortex, are filled with myxomatous tissue, in greater part, in addition to some marrow cells and a thin layer of osteoblasts.

The cortex, on its surface, sawcuts, and body, exhibits great absorption being riddled with osteoclasts and having its spaces filled in as stated above. Its canals communicate freely with those of the periosteal trabeculæ. In the medullary cavity there are the remains of osteoid trabeculæ which show considerable absorption. Osteoblasts are no longer present. The marrow is red on the edges and yellow in the centre. There are osteoclasts in its substance.

Experiment 67. Operation performed January 26, 1906. Animal killed March 5, 1906. Fracture of thirty-eight days' duration.

Macroscopic Description. The wound had healed by first intention. Palpation revealed some roughening at the site of the operation, with two especially hard and nodular places, giving the impression that the catgut had failed to absorb. On removing the skin the tissues and the two nodules mentioned above were found to be freely movable over the bone.

Longitudinal section through the bone showed some thickening over the site of the injury to the bone, but there was no apparent callus. The medulla did not appear to be affected.

Microscopic Description. The periosteum exhibits some proliferation of its deeper layer. The injury to the bone is filled with young osteoid trabeculæ which have wide medullary spaces and are covered with osteoblasts. Their general direction is indefinite, but more in the direction of the bone than otherwise. The tissue in the spaces is of a loose cellular type, but not myxomatous in appearance. There is no absorption of the trabeculæ.

Beyond where the periosteum was sewed together there is a thin layer of compact new bone. The direction of the spaces in it are not oblique as in other instances, but more longitudinal. The catgut is present and encapsulated with fibroblasts. There is superficial absorption of the cortex by osteoclasts. The cortex proper shows very slight absorption, all of which is in the neighborhood of the periosteum. Its canals contain some osteoclasts, marrow cells, and doubtful osteoblasts. A few of the canals communicate with the spaces of the new bone formation. The medulla is cellular at the edges and yellow at the centre. There are no osteoid trabeculæ in the medullary cavity. The marrow contains some osteoclasts.

Experiment 68 Operation performed January 26, 1906. Animal killed March 16, 1906. Fracture of forty-nine days' duration.

Macroscopic Description. The wound had united by first intention. On palpation through the skin, a small nodule could be felt at the site of the operation upon the bone. On removal of the skin the nodule appeared to be attached to the bone. In addition, there was found what seemed to be an unabsorbed strand of catgut. There was very little callus found at the site of operation.

Longitudinal section through the bone showed slight thickening of the periosteum at the site of the operation, but no callus. The medulla, apparently, was not affected, being light pink in color.

Microscopic Description. The catgut is undergoing absorption by leukocytes and a few giant cells. The periosteum appears to be mostly fibrous in nature, having ceased most of its activity. The bone wound is filled in with quite compact osteoid tissue, the general direction of the trabeculæ being in the line of the bone. Its spaces communicate with the old Haversian spaces of the cortex. The trabeculæ are still covered with osteoblasts which do not appear to be very active. There is absorption of its superficial portions.

Excluding superficial absorption, the cortex shows practically no rarefaction, but looks as if it had undergone absorption in places, and had been refilled with new bone. This impression is given by the different shades of staining in the cortical bone.

The medullary cavity contains a great number of osteoid trabeculæ which are rather dense. They are covered with osteoblasts, but no absorption is apparent. The medulla is red near the trabeculæ and shows an increase in its connective tissue. It contains some osteoclasts.

SERIES 7.

This series, upon dogs, was performed with the object of determining whether an animal substance placed under the periosteum would cause the formation of bone when not associated with injury to the bone. Each operation consisted in making an incision over the tibia to the bone. A longitudinal incision was then made along the side of the bone and the periosteum was raised up. A coil of catgut, No. 2, was placed under it and the wound closed with silk and covered with collodion.

Experiment 69. Operation performed June 7, 1906. Animal killed July 12, 1906. Fracture of thirty-five days' duration.

Macroscopic Description. The wound had united by first intention. Removal of the skin revealed the fact that the catgut remained unabsorbed. No callus or new bone could be noticed. Section through the bone showed the periosteum to be greatly thickened and the unabsorbed catgut to be encapsulated. There was no apparent new-bone formation. The medulla was red in the upper half of the section and yellow in the lower. In the medullary cavity there was no callus or new-bone formation.

Microscopic Description. The catgut is encapsulated, with slight absorption taking place by the action of leukocytes, which are rather abundant. The encapsulating tissue consists of fibroblasts with large vesicular nuclei. At one place, where the periosteum is still attached to the bone, there is a thin deposit of dense osteoid tissue which exhibits slight absorption. The trabeculæ are not covered by osteoblasts. There is superficial absorption of the cortex by osteoclasts. In the cortex proper, near the periosteal surface, there is some absorption with widening of a few of the Haversian spaces. These are lined by osteoclasts. The medulla shows increased cellular content on the edges. No osteoclasts or trabeculæ are present.

Experiment 70. Operation performed May 29, 1906. Animal killed July 19, 1906. Fracture of fifty-one days' duration.

Macroscopic Description. The wound had healed completely without suppuration. On removing the skin, the catgut was found coiled up as when it had originally been placed under the periosteum and no absorption was apparent.

The catgut was adherent but the periosteum was not thickened and there did not appear to be any new-bone formation. Longitudinal section showed no new-bone formation, but the entire marrow was of the red variety. No microscopic sections were made owing to the fact that the gross specimen was mislaid.

SERIES 8.

This series, upon dogs, was performed with the object of determining whether an unabsorbable substance placed under the periosteum would excite the formation of bone when not accompanied by injury to the cortex.

The operations were similar to the preceding one, with the exception that celloidin, hardened in bichloride alcohol, was placed under the periosteum instead of catgut. In this series, as in the two series following, there is but one successful operation.

Experiment 71. Operation performed May 29, 1906. Animal killed July 19, 1906 Fracture of fifty-one days' duration.

Macroscopic Description. The wound had united perfectly without suppuration. No thickening could be felt at the site of the operation. On removing the skin the celloidin was found still in the place where it had been put, but dried out and brown in color. It was not encapsulated and the periosteum did not appear to be thickened. There was no visible bone formation.

Longitudinal section confirmed the above. The medulla was red throughout the bone.

Microscopic Description. The celloidin still remains, but it appears as if it had slipped out from under the periosteum, as there is periosteum between it and the bone. There is an apparent effort at encapsulating the celloidin, a layer of fibroblasts and leukocytes being around it. The periosteum shows proliferation in its deeper surface and there is superficial absorption of the cortex by osteoclasts. There is no sign of trabeculæ formation. The cortex proper has undergone no absorption. The medulla is red and contains osteoclasts.

SERIES 9.

This operation was performed with the object of determining the influence of a blood clot underneath the periosteum when not associated with injury to the bone itself. It consisted in making an incision over the radius to the bone and raising the periosteum. The periosteum was very thin and it was difficult to elevate it. The wound was then sutured without any effort having been made at hemostasis in the deeper parts of the wound.

Experiment 72. Operation performed June 7, 1906. Animal killed July 19, 1906. Fracture of forty-two days duration.

Macroscopic Description. The wound had healed completely without suppuration. On removing the skin there was found a small, discolored area under the wound in the soft tissues, but no evidence of the operation upon the bone could be found.

Longitudinal section failed to reveal any evidence of the operation.

Microscopic Description. There is superficial lacunar absorption of the cortex by osteoclasts, where the periosteum had been raised. There is no attempt at trabeculæ formation. The deeper layer of the periosteum exhibits some cellular proliferation, the superficial layer still being fibrous. The cortex proper shows no absorption whatever. The marrow is red in both the injured and uninjured bone, and there are osteoclasts throughout its entire substance.

SERIES 10.

This operation was performed with the object of determining the effect upon bone production of merely raising the periosteum and letting it immediately fall back into place. An attempt at perfect hemostasis was made. The operation was similar to Experiment No. 72 in every other respect.

Experiment 73. Operation performed June 7, 1906. Animal killed July 19, 1906. Fracture of forty-two days' duration.

Macroscopic Description. The wound had healed completely without suppuration. On removing the skin there was found a small patch of discoloration under the wound in the soft tissue, but there was no evidence of the operation upon the bone. Longitudinal section through the bone failed to reveal anything further.

Microscopic Description. There is superficial absorption of the cortex by osteoclasts and proliferation of the deeper layers of the periosteum, with no attempt at trabeculæ formation. The unoperated bone, also, shows slight absorption of its surface, but to a very minor degree. In the cortex there are several dilated spaces caused by absorption by osteoclasts. These spaces are lined by osteoclasts. The marrow of both bones is red and shows osteoclasts.

SERIES 11.

This series consisted of two operations upon dogs and had as its object to determine whether a subperiosteal resection, when not in the presence of infection, would be followed by complete regeneration of the bone. This series did not consist of a simple complete subperiosteal resection, but differed in that a thin strip of bone was left remaining in order to see if it would hasten the regeneration by allowing a framework upon which to build.

Each operation was performed by making an incision over the tibia to the bone, and then a longitudinal incision through the periosteum for about two inches. The periosteum was raised from the bone for almost the entire circumference and then retracted, while two sawcuts, about an inch apart, were made half-way through the bone. The intervening portion of the cortex was grasped between the jaws of a bone forceps and a lid taken off the medullary cavity between the two cuts. The remainder of the bone intervening was removed piecemeal by bone forceps, with the exception of a thin strip next to the fibula. The cut ends of the tibia were bevelled off as much as possible to avoid leaving a dead space when the periosteum was sewed up. The periosteum was then sutured throughout the entire length of the incision in it, so that the two inner surfaces were in contact. Chromicized catgut was used for the purpose. The skin was also sutured with a buttonhole suture of chromicized catgut and the leg wrapped in a wet bichloride dressing. Finally, the leg was placed in a shaped splint, which was retained in position with a sodium silicate dressing.

Experiment 74. Operation performed November 19, 1906. Animal killed January 5, 1907. Fracture of forty-seven days' duration.

Macroscopic Description. The wound had healed perfectly and the leg was firm. There was no catgut remaining. A large nodular callus filled in the defect in the tibia. It appeared as if there had been a fracture of the fibula that had reunited.

Radiograph (Fig. 28). The *x*-rays reveal the fact that the fibula and the remaining strip of tibia had evidently been fractured, but the position of the fragments had remained good and there is no apparent shortening. The areas from which the cortex had been removed are still visible. There is what appears to be a small deposit of osteoid tissue on one fragment.

Longitudinal section through the bone confirms the *x*-rays in that a fracture of the fibula with callus was found. The defect in the tibia was filled with cartilage, with a small amount of osteoid tissue at the ends of the cortex of both fragments.

Microscopic Description. The sections confirm the x-rays and gross findings. The fibula is fractured and the site of fracture is surrounded by a spindle-shaped callus of osteoid tissue, with a wide band of fibrocartilage across the line of fracture. The portion of the tibia from which the cortex and medulla have been removed is filled with a dense fibrocartilage. The parts of the cartilage nearest the ends of the fragments appear more hyaline, the cells becoming larger and the intercellular substance less fibrillated. The cells arrange themselves in rows resembling the epiphysis of a growing bone. The ground substance between these rows is absorbed and vascularized. This is followed by a deposition of calcium salts and the formation of osteoid trabeculæ over the exposed medullary ends of the fragments. Between the trabeculæ are large, thin-walled bloodvessels.

The old cortex has undergone considerable absorption, especially at the operated ends, where the lacunar osteoclastic absorption is distinctly evident. The medulla is somewhat cellular, but many fat cells remain.

Experiment 75. Operation performed November 22, 1906. Animal killed January 5 1907. Fracture of forty-four days' duration.

Macroscopic Description. The wound was healed perfectly. On removing the skin all signs of the catgut had disappeared. The leg was firm, the animal having had use of it. A large nodular callus filled in the interval where the bone had been removed from the tibia. This callus was thicker than the original bone.

Radiograph (Fig. 29). *X*-rays reveal the fact that the fibula had remained unbroken, but the strip of tibia that had been left remaining had been fractured, but was retained in perfect position. This strip of old cortex is covered with new-bone formation. This deposit does not fill in the entire defect in the tibia, but is only about one-eighth inch thick at its thickest portion. There is still another deposit of osteoid tissue separated from this mass in the form of an island between the two fragments of bone. The remainder of the defect must be filled in with cellular material, as it appears in the picture as a mere haze.

Longitudinal section confirms the *x*-rays completely and shows that the material filling in the defect is cartilage with the exception of the osteoid tissue before mentioned.

Microscopic Description. The fibula is intact, but has a deposit of osteoid tissue upon its tibial aspect for almost the entire length of the bone.

The defect in the tibia is filled by cartilage and osteoid tissue. The osteoid trabeculæ are along the bridge of old bone left remaining between the two fragments, across the ends of the bone and in the form of a small island in the cartilage filling in the remainder of the defect. The cartilage is more fibrous in nature than hyaline. At its edges the cells have a tendency to arrange themselves in rows, but to a lesser degree than in the last experiment.

The osteoid trabeculæ, as in most of the following experiments, appear to be composed of very delicate trabeculæ caused by direct transformation of cartilage by a deposition of calcium salts. These form the interstitial bone and take a bluish stain. Upon the surface of these there is a deposit of new bone which is much denser in appearance and takes a deep-red stain with eosin. This tissue has a distinct lamellar arrangement and resembles osseous tissue, forming Haversian canals and systems.

The cortex has undergone great absorption, with a deposition of new bone in the widened spaces. There is lacunar absorption of the ends of the bone. The marrow does not appear to be very cellular.

SERIES 12.

This series, upon dogs, was performed with the object of determining if there would be regeneration of bone after a complete subperiosteal resection when not in the presence of inflammation. There were two operations in this series and each was identical with the previous two, with the exception that there was no bridge of cortex left, but the entire section of bone removed and the periosteum then sutured as before.

Experiment 76. Operation performed November 26, 1906. Animal killed January 11, 1907. Fracture of forty-six days' duration.

Macroscopic Description. The wound had healed by first intention. There was some rocking mobility and it appeared as if the fibula had been fractured, as there was some callus in that region. The catgut was still present, but encapsulated. There was some thickening in the region of the operation, but there did not appear to be regeneration of the bone.

Radiograph (Fig. 30). *X*-rays reveal the fact that the fibula had been broken and that there was lateral deviation of one of the fragments of the tibia. There appears to be a slight new-bone formation at the ends of the tibia, but none whatever in the space where the cortex had been removed. There is some osteoid callus at the site of the fracture of the fibula.

Longitudinal section was very unsatisfactory, not having gone through the centre of the bone, so that no conclusions could be drawn from it.

Microscopic Description. The fibula is not to be found owing to the direction of the sections and the half of the bone chosen for section. The area from which the cortex had been removed is filled in greater part by very dense connective tissue with fibrocartilage near the ends of the tibia. The medullary cavities of the exposed ends are covered in with a very thin layer of osteoid tissue. There is also a small nodule on the fibular aspect of the periosteal side of one of the fragments. A rather large island of osteoid tissue is on the fibular side of the space from which the bone had been removed. This is probably a portion of the callus of the fractured fibula.

The callus present is undergoing absorption principally by leukocytes.

The cortex is greatly rarefied and the ends of the fragments exhibit extensive lacunar absorption by osteoclasts. The marrow is rather cellular.

Experiment 77. Operation performed November 28, 1906. Animal killed January 11, 1907. Fracture of forty-four days' duration.

Macroscopic Description. The wound had healed perfectly. The leg was quite firm, but there was some rocking motion to be felt at the site of the operation. The catgut was still present and encapsulated. The defect in the tibia was filled in comparatively well and was rather firm, but not bony.

Radiograph (Fig. 31). *X*-rays reveal the fact that the fibula had been fractured and was in quite good position at the time the picture was taken. There is a moderate amount of callus thrown out around the site of the fracture. The tibia has shortened, closing in the space between the ends of the bone and obliterating the defect excepting where the bone is bevelled off. There is no new bone where the old had been removed.

On the bevelled surface of one fragment there is a very thin deposit of new bone.

On longitudinal section the fibula was shown to have been fractured. There was a comparatively large callus thrown out around the line of fracture. The defect in the tibia was still present and did not appear to be filled in with anything but fibrous tissue, which was not very dense. On the periosteal surface of the ends of the fragments there was some spongy bone formation, and there was also a thin shell of osteoid bone formation over the ends of the medullary cavities. The marrow was red in color.

Microscopic Description. The sections confirm the *x*-ray and gross findings. The defect was filled in with rather cellular fibrous tissue. There is an osteoid nodule from the fibular callus which is surrounded by fibrocartilage. The medullary cavities of the ends of the fragments of the tibia are covered by osteoid trabeculæ which are undergoing lacunar absorption in areas. The spaces between them are filled with very cellular marrow. The cortex is riddled with dilated Haversian spaces. The catgut can be seen undergoing absorption.

SERIES 13.

This series, consisting of two operations upon dogs, was performed with the object of determining if the placing of medullary tissue between the two surfaces of the periosteum, after removing the cortex, would influence the regeneration of the bone. The operations were identical with those of the previous series, with the following exception. When the lid of the cortex was removed from between the two sawcuts, the medulla was removed intact and placed in normal salt solution while the remainder of the cortex was being removed.

It was then placed between the two layers of periosteum before they were sutured together.

Experiment 78. Operation performed December 26, 1906. Animal killed January 26, 1907. Fracture of thirty-one days' duration.

Macroscopic Description. The wound had united by first intention. On removing the skin the catgut was found to have been absorbed with the exception of the knot. The leg was in good alignment and quite firm. A very slight amount of rocking could be elicited but no mobility at the site of operation.

Radiograph (Fig. 34). Unfortunately the picture was not taken at quite the right angle, so that the tibia is partially in front of the fibula, but, nevertheless, every point is distinct. The fibula is seen to be intact and there is no shortening of the tibia, the ends of the fragments remaining separated as at the time of the operation. There is a bridge of osteoid tissue extending from one fragment to the other, the thickness of which cannot be determined because of the angle of the picture.

Longitudinal section confirmed the radiograph. The defect was filled with cartilage containing islands of rather dense bone; also more spongy bone. The marrow of the ends of the fragments was red, but the medullary cavities did not appear to contain much new-bone formation with the exception of the ends, which were closed in by it. The periosteal aspect did not seem to have any new-bone formation upon it.

Microscopic Description. The fibula is intact and has upon its periosteal surface a deposit of new bone. The cortex is greatly rarefied.

The defect in the tibia is filled in with irregular masses of hyaline and fibrocartilage, osteoid tissue, and dense fibrous tissue. The osteoid tissue is quite abundant. Its marrow spaces are very large and filled with cellular red marrow and red blood cells. The cartilage is mostly of the very cellular hyaline variety, taking a deep-blue hematoxylin stain. There is also some fibrocartilage and dense fibrous tissue which is becoming riddled by bloodvessels. The old cortex shows great absorption. The marrow is somewhat cellular, and, extending across the medullary cavity at the ends of the fragments, there are bridges of osteoid trabeculæ.

Experiment 79. Operation performed November 30, 1906. Animal killed January 21, 1907. Fracture of fifty-two days' duration.

Macroscopic Description. The wound had healed by first intention. The leg was perfectly firm and solidly united without a depression at the site of operation. On removing the skin the catgut was found to have been absorbed, and there was a slight elevation at the site of the original defect in the tibia.

Radiograph (Fig. 35). This is very interesting and in marked contrast to those of the other series. The fibula is seen to be intact and the distance between the ends of the tibia about the same as at the time of operation. The defect in the tibia is more than refilled with new-bone formation, which is continuous from one fragment to the other and wider than the normal width of the bone. It shows some light streaks through the mass, due probably to islands of cartilage.

Longitudinal section through the bone confirmed the radiograph in all respects. The new bone was found to be vascular, red, spongy bone. It was broken into large islands by the interposition of narrow islands of cartilage. The marrow of both fragments was red and hyperplastic, but there did not appear to be a great amount of new-bone formation in either of the ends of the bone.

Microscopic Description. The fibula is intact, but has shared in the general regenerative process, and the cortex has undergone considerable rarefaction. Its periosteum has proliferated and osteoblasts are present in abundance.

The defect in the tibia is filled in completely by osteoid tissue and cartilage. The trabeculæ are arranged in irregular anastcmosing masses with cellular hyaline cartilage, taking a deep-blue hematoxylin stain on the outskirts. Between the masses are slits or spaces lined on both sides by cartilage, probably due to the fact that the masses began as separate islands of cartilage which grew until they were in apposition with each other, but not united. The spaces between the osteoid trabeculæ are quite large and filled with very cellular red marrow and red blood cells. The trabeculæ are covered with osteoblasts, and there are a great number of very large osteoclasts present, causing absorption in other areas. The old cortex is riddled with dilated Haversian spaces. The marrow is cellular toward the ends of the fragments and contains the variously sized cells, etc., described under several previous experiments. Near the site of the operation it contains numerous multinuclear cells indistinguishable from osteoclasts. The trabeculæ are composed of osteoid tissue, evidently due to calcification of the old cartilage, on which have been deposited several lamellæ of osseous tissue, taking a deep eosin staining.

SERIES 14.

This series consisted of two operations upon dogs with the object of determining whether or not placing dried bone chips between the two layers of the periosteum, after removing the cortex, would hasten the regeneration of the bone. The operations were identical with those of Series 12, with the exception that before sewing the periosteum, thin slices of bone that had been ground and the balsam removed were placed between the two layers of periosteum and sewed there.

Experiment 80 Operation performed December 28, 1906. Animal killed January 5, 1907. Fracture of eight days' duration.

Macroscopic Description. The wound was healed and the sutures were still in place. The sections of bone placed between the layers were still there and surrounded by blood clot.

Radiograph (Fig. 32). *X*-rays reveal the fact that the fibula was not fractured and that there was no shortening of the space between the ends of the tibia. Slight evidences of the thin strips of bone that had been placed between the layers of the periosteum, can be seen, but they do not come out as plainly in the prints as they do in the negative. There is no apparent growth of new bone anywhere.

Longitudinal section demonstrated that the defect in the bone was filled with firm blood clot and that the medulla of the ends of the fragments was firm and condensed. It was red in color.

Microscopic Description. The fibula is not fractured, but is rarefied and has a deposit of osteoid tissue upon its periosteal surface. In the centre the defect in the tibia is filled with blood clot. Toward the ends of the fragments of the tibia it is replaced by loose connective tissue. Throughout this tissue, which appears to take origin from the medulla, are numerous round or oval cells with round, well-staining nuclei, and a considerable amount of protoplasm. These cells resemble closely the normal marrow cells. The tissue is quite vascular.

The marrow of both fragments exhibits considerable connective-tissue hyperplasia and contains abundant, thin-walled, engorged bloodvessels, which are streaming into the tissue filling the defect in the bone. The cortex has undergone extensive absorption, especially at the ends. There are abundant osteoid trabeculæ on the periosteal surface, also in the medullary cavity near the ends and over the ends of the cortex itself. The catgut is still plainly visible, encapsulated by connective tissue and leukocytes, not having undergone absorption.

Portions of the strips of bone placed between the layers of periosteum can be seen. Some of them appear to be undergoing lacunar absorption. There is no deposit of new bone on the surface, as was seen in many of the experiments where the splinter of bone had been broken off at the time of the operation.

Experiment 81. Operation performed December 28, 1906. Animal killed January 26, 1907. Fracture of twenty-nine days' duration.

Macroscopic Description. The wound had united, but the fibula was fractured and there was no attempt whatever at union. A large nodule could be felt over the fibula. The catgut remained unabsorbed and the defect in the tibia showed no evidence of new-bone formation.

Radiograph (Fig. 33). *X*-rays reveal the fact that there was a fracture of the fibula, with angular displacement and non-union. Callus can be seen above and below the site of fracture of the fibula. The space in the tibia from which the bone had been removed has collapsed. There is no apparent new-bone formation in it and no signs of the piece of bone placed between the layers of the periosteum.

Longitudinal section confirmed the x-ray findings The defect in the tibia was as when it was made with the exception that the alignment of the tibia had changed by displacement and this reduced the length of the defect. It was filled in with soft connective tissue. No bone or cartilage was to be seen. Along the sides of the medullary cavity of the ends of the fragments there was some new-bone formation and also on the periosteal surface. The medulla was pink in color.

Microscopic Description. The fibula is fractured and there is considerable callus in the region of the fracture, upon both fragments, with cartilage across the line of fracture.

The defect in the tibia is filled with loose, vascular, cellular connective tissue. But few osteoid trabeculæ are present, these being upon the fragments of the tibia. There are no signs of the section of bone placed between the layers of the periosteum. There is considerable absorption of the cortex of both fragments, interstitially and at the ends. The marrow is somewhat hyperplastic.

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